



## Spatio-temporal abundance and dispersal of Culicoides

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# **Spatio-temporal abundance and dispersal of *Culicoides***

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1870 Frederiksberg C 2013  
VET-PHD-2013

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All photos and graphics by Carsten Kirkeby unless otherwise stated.

Front cover: The author under a CDC light trap; model graphics; a biting midge  
biting the Author's father.

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# Summary

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This PhD project comprises studies of biting midges (*Culicoides*) in Denmark with regards to vector-borne diseases such as bluetongue virus (BTV) and Schmallenberg virus (SBV). Both diseases are new in northern Europe. In Denmark there was an outbreak of BTV in 2007 and 2008. BTV infects ruminants, and especially infected sheep and cattle constitute a problem for farmers. The symptoms of BTV include fever, cyanotic tongue, oedemas and decreased milk production. The last symptom affects the economy and animal welfare in the farming industry. In 2011 and 2012, outbreaks of SBV were also recorded in Denmark. The symptoms of SBV are similar to BTV but also include a high proportion of malformations and stillbirths in lambs.

Models of vector-borne diseases can be used to predict an outbreak and evaluate e.g. the optimal control strategy, the economic impact and the number of infected animals. These models need to have proper input regarding the abundance and behavior of the vectors. If no vectors are present in an area, the disease will not spread. Thus the vector abundance is a very important factor for models of vector-borne diseases. This PhD project investigates different key factors important for the abundance and behavior of vectors. There are four different parts of this project:

## Vector abundance

The abundance of *Culicoides* vectors in an area is often estimated using light traps. In the first study of the present project, light traps were used to sample

a field with sheep for vectors with regards to different factors determining the vector abundance. The result was a spatial statistical model where the distance to sheep was found significant for the vector abundance. Some temporal factors were also found significant: Temperature, wind speed, humidity, wind turbulence and precipitation. These significant factors can be used in simulation models for vector-borne diseases to estimate the vector abundance. Furthermore, an unexplained pattern was found in this study: There were significant clusters of higher vector abundance found in different sites every night on the field. This pattern might be explained by swarming behavior. The implications of this is that one trap can not be regarded as representative for the vector abundance on a field because the vector abundance can be up to 11 times higher within a cluster than outside.

## Optimized sampling

In the second study of the project the light trap catches from the first study were explored in order to determine the optimal sampling strategy. It was tested if a better estimate of the vector abundance could be obtained by separating traps spatially. There was no indication of this. Furthermore, the risk of falsely detecting absence of vectors on the field was explored. The result was that a more optimal sampling could be obtained with spreading the trap catches in time rather than space.

## The range of attraction of light traps

The third study in this project was conducted to determine the range of attraction for the light traps. The traps were set up in two different configurations to collect data for testing three different models of how the vectors are attracted to the light traps. The models were fitted to the data, and the best model was found. The result showed that the vectors are able to evaluate light sources in the horizon and fly towards the strongest. The range of attraction for the 4 W CDC type light trap was estimated to be 15.25 m.

## Dispersal of vectors between farms

In the fourth study of this project, a new technique of marking and detecting *Culicoides* was developed. This new technique does not require anaesthesia of the specimens while marking, and the detection method was objective. Mark-recapture studies have not previously been carried out in the field in Europe although the dispersal behavior is an important factor in models for vector-borne diseases. 29% (eight specimens) of the recaptured specimens of the *Pulicaris* group were caught at a pig farm 1750 m away from the release point. Only two specimens of the *Obsoletus* group were recaptured and they were caught in the release point. The most important result is that the eight *Pulicaris* specimens were caught upwind from the release point. This shows that *Culicoides* vectors are able to seek hosts upwind. This result is important when modeling the spread of vector-borne diseases transmitted by *Culicoides*.



# Resumé

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Dette PhD projekt omhandler studier af mitter i Danmark i forbindelse med vektorbårne sygdomme. I 2007 og 2008 havde vi i Danmark udbrud af bluetongue virus (BTV) som spredtes med mitter. BTV rammer drøvtyggere som f.eks. køer og får, og symptomerne er bl.a. feber, ødemer, blåfarvning af tungen samt nedsat mælkeydelse. I 2011 og 2012 blev der desuden i Danmark konstateret den nyligt opdagede Schmallenberg virus (SBV) der ligesom bluetongue spredtes med mitter til drøvtyggere. Symptomerne på SBV ligner symptomerne på bluetongue, men inkluderer desuden en stor forekomst af deforme og dødfødte lam. For at kunne teste forskellige scenarier med henblik på kontrol og udryddelse af sådanne sygdomme er det nødvendigt at have præcise epidemiologiske modeller som kan benyttes til at undersøge den optimale strategi før, under og efter et udbrud. Disse modeller bygger på parametre som beskriver mitternes adfærd og forekomst. Det f.eks. er afgørende hvor mange mitter der er i et område. Hvis der er mange mitter spredes sygdommen hurtigere og mere effektivt end hvis der er få. I dette PhD projekt er forskellige nøgleparametre for mitternes adfærd undersøgt. Projektet består af fire forskellige studier som er beskrevet herunder.

## Mitternes tæthed

Mitternes antal estimeres ofte ved hjælp af lysfældefangster. I det første studie i dette projekt undersøgte mønsteret af lysfældefangster på en mark med får. Dette blev undersøgt i relation til forskellige faktorer som f.eks. ynglesteder og afstand til værtsdyr. Resultatet blev en spatiel statistisk model hvor afstanden til værtsdyr var afgørende for hvor mange mitter der blev fanget i lysfælderne.

Desuden blev flere temporale faktorer fundet afgørende for lysfældefangsterne: Temperatur, vindhastighed, fugtighed, turbulens og nedbør. De afgørende parametre kan bruges i simuleringsmodeller til at estimere mitternes antal. Derudover blev i dette studie opdaget et hidtil ukendt mønster, hvor mitterne på forskellige nætter samledes i større antal (clusters) forskellige steder på marken. Dette mønster kan bedst forklares af sværmning, og betyder at en fælde ikke nødvendigvis er repræsentativ for et område, da fangsten indenfor et cluster kunne være op til 11 gange højere and udenfor.

## Optimal sampling

I det andet studie blev lysfældefangsterne fra det første feltstudie undersøgt for at finde den optimale strategi for sampling med lysfælder. Resultatet var at et bedre estimat af mitternes tæthed på en given nat ikke blev mere præcist af at placere fælderne med større afstand. Dette var ellers forventet p.g.a. fundet af clusters. Resultatet er relevant for mange monitoringsstudier, hvor man bruger lysfælder til at estimere antallet af mitter i et område. Desuden bliver fælderne brugt til at konstatere om der overhovedet er mitter i et område, f.eks. i forbindelse med bestemmelse af den vektorfri periode om vinteren.

## Lysfældernes fangstområde

I tredje studie blev lysfældernes fangstområde undersøgt. Lysfælderne blev stillet op på en mark i to forskellige konfigurationer for at samle data til modellering af deres fangstområde. De to datasæt blev brugt til at fitte 3 forskellige modeller for mitternes tiltrækning til lysfælderne. Resultatet viste at mitterne ser lysfælder på en ikke tidligere beskrevet måde, hvor de vurderer lyskilder i horisonten og flyver mod den stærkeste koncentration af lys. Forsøget viste desuden at den benyttede type lysfælde med et 4W lysstofrør havde en rækkevidde på indtil 15.25 m afstand.

## Mitternes spredning mellem gårde

I det fjerde studie udviklede jeg en ny metode til at mærke og genfange mitter for at kunne følge deres spredning direkte. Denne metode blev udviklet til formålet

og udmærker sig ved at virke uden bedøvelse af mitterne samt at mærkede mitter kan detekteres objektivt. Fangst-genfangst forsøg er ikke tidligere blevet udført på mitter i Europa, selvom det er vigtigt at undersøge, da spredningsmodeller bruger lokal spredning som et parameter. Der blev mærket og udsat 1460 mitter, hvoraf 30 individer blev genfanget. Et af de interessante resultater var at i løbet af to nætter blev 29% af de mærkede mitter i den ene artsgruppe, *Pulicaris* gruppen, fanget 1750 m væk, op imod vinden, på en gård med grise. Da 97.5% af kvægbesætninger i Danmark ligger indenfor en afstand af 1600 m til den nærmeste besætning, viser forsøget at mitterne er i stand til at sprede vektorbårne sygdomme mellem dem effektivt.



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# Preface

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Before I started on this PhD project, I worked for two years with *Culicoides* in the Section of Veterinary Epidemiology at the DTU Veterinary Institute. A new wave of *Culicoides* research had begun after the outbreak of bluetongue virus in northern Europe in 2006. Among other activities in the national surveillance program for *Culicoides*, I put up light traps on farms across Denmark in order to determine the vector free period. From this work I soon found out that the trap catches vary greatly spatially and temporally.

This initiated my interest in the behavioral patterns of vectors and epidemiology in general. During the past three years I have found many answers, but with those followed even more new interesting questions.

Copenhagen, January 2013

A handwritten signature in black ink, appearing to read 'Carsten Kirkeby', with a stylized, cursive script.

Carsten Kirkeby



# Supervisors

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**Main supervisor:**

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**Co-supervisor:**

Anders Stockmarr  
Senior Researcher  
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DTU Compute  
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# Papers included in the thesis

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## 1 Appendix A

**Carsten Kirkeby**, René Bødker, Anders Stockmarr & Peter Lind  
Spatial abundance and clustering of *Culicoides* (Diptera: Ceratopogonidae) on a local scale.

In press, *Parasites & Vectors*, 2013.

## 2 Appendix B

**Carsten Kirkeby**, René Bødker, Anders Stockmarr & Peter Lind  
Spatio-temporal optimization of sampling of *Culicoides*.

Submitted to *Parasites & Vectors*, 2013

## 3 Appendix C

**Carsten Kirkeby**, Kaare Græsbøll, Anders Stockmarr, Lasse Engbo Christiansen & René Bødker

The range of attraction for light traps catching *Culicoides* biting midges (Diptera: Ceratopogonidae).

In press, *Parasites & Vectors*, 2013.

## 4 Appendix D

**Carsten Kirkeby**, René Bødker, Anders Stockmarr, Peter Lind & Peter M. H. Heegaard

Quantifying dispersal of European *Culicoides* (Diptera: Ceratopogonidae) between farms using a novel mark-recapture technique.

Submitted to *PLoS One*, 2013



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I especially thank Kaare Græsbøll for being a great collaborator, colleague and friend.

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I would also like to thank my student workers; Astrid Block van Witteloostuijn, Josepha Axelsen, Simon Haarder and Peter Iversen for great help in the laboratory.

I also thank lab technicians Heidi Gertz Andersen and Jeanne Toft Jakobsen for help in the immunology lab.



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I thank Frank Hansen and Rune Ploug from Vallø Lam for invaluable help with managing sheep and gear in the field, Peter Henrik Tesdorpf and Christian L. Pedersen at Gjorslev for permission and help with field setup, Anker Jørgensen on Rødvigvej for lending me the tractor and stables for experiments, and Jens Jensen in Esrum for kind permission to catch biting midges for lab studies.

I thank Mikkel Brydegaard for the inspiring work on new methodologies and expanding the scientific horizon. I hope we will be able to collaborate in the future.

I thank the former generation of *Culicoides* researchers that I have had the pleasure to meet and learn a lot from; Søren Achim Nielsen, Rudy Meiswinkel and Jean-Claude Delécolle. I also thank Sandra Lassen for collaboration during the project.

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## CHAPTER 1

# The dawn of epidemiology

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## 1.1 The first spatial cluster

In 1849, the physician John Snow investigated an outbreak of cholera in Soho,  
5 London. By plotting the deaths related to cholera on a map, he discovered a spatial cluster of deaths around a water pump in Broad Street (Fig 1.1). It was the first time an outbreak of a disease was investigated in this way. The pump led contaminated water in from the river Thames. John Snow had the handle removed from the pump and the outbreak ceased. This manoeuvre made him  
10 the founding father of modern epidemiology.

After more than 160 years, epidemiology today is still about revealing patterns in space and time. Although many tools for this have been developed in the meantime, the purpose is still the same. It can be to investigate periodicity in some data on a temporal scale, or to find clusters in spatially resolved data. In  
15 this PhD project the spatial and temporal pattern of the abundance of biting midges, *Culicoides*, is investigated, and factors that could explain the observed pattern are tested.

Biting midges are some of the most annoying creatures in the world. They can  
20 appear out of nowhere in enormous numbers, painfully biting and sucking the blood of every warm-blooded creature on their way. For the very same reasons



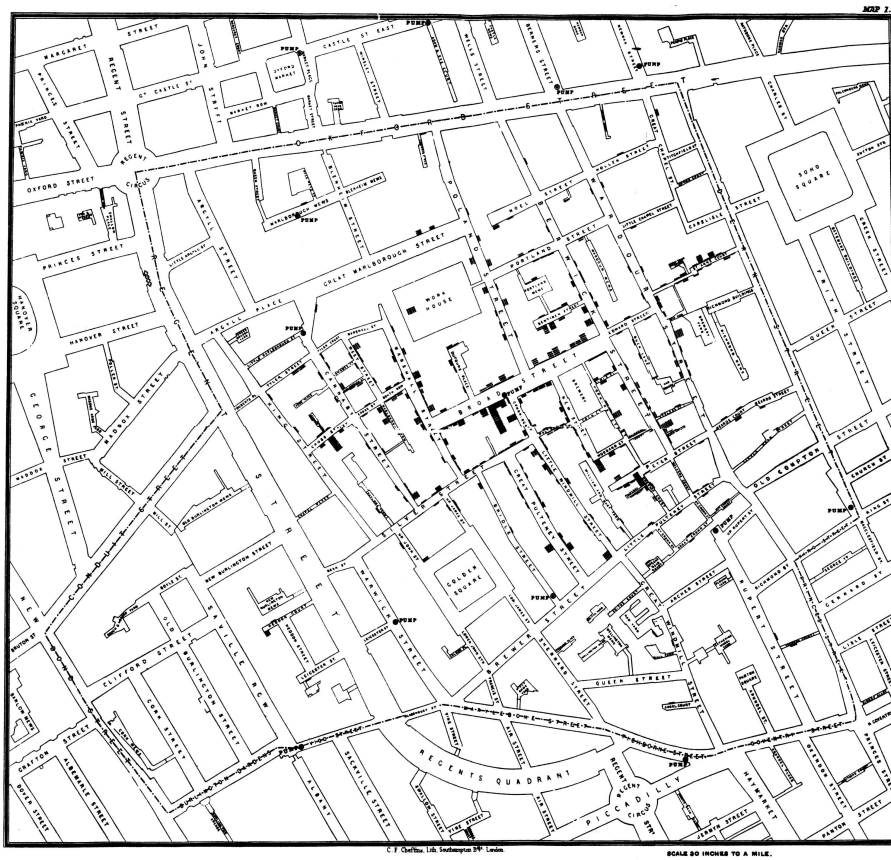


Figure 1.1: The original map by John Snow showing the deaths (marked with black) caused by a cholera outbreak in London, 1854. He wrote: "On proceeding to the spot, I found that nearly all the deaths had taken place within a short distance of the pump." [120]. The drawing is public domain.

they are annoying, they are interesting in a scientific perspective. They suck blood from a variety of hosts in almost all parts of the world [87], transmitting numerous diseases between the hosts. Therefore they are an important topic of research. This PhD project contribute to our understanding of the world of *Culicoides* on a basic and applied research level, quantifying important measures for use in epidemiological simulation models and revealing new knowledge about their behavior.



## CHAPTER 2

# Global swarming

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30

## 2.1 The biting midges

Some of the greatest entomologists have been hatched in Denmark, when it comes to *Culicoides*. In 1839, the dane R. C. Stæger described *Culicoides pic-*  
*tipennis* (Stæger), *C. fascipennis* (Stæger) and *C. vexans* (Stæger). His type  
35 specimens are today kept at the Natural History Museum in Copenhagen. To-  
day we still have some of the finest *Culicoides* researchers in Denmark: Boy  
Overgaard Nielsen and Søren Achim Nielsen have contributed to the World's  
knowledge on *Culicoides*, and have investigated their act as a public nuisance  
[95], their preferred area on the host to bite [94], species differentiation [97], and  
40 their activity and seasonal dynamics [96], to mention a few. Recently, Sandra  
Lassen has explored the host preferences [80] and species differentiation [81]  
of *Culicoides*. If we look further out in Europe, other researchers are working  
hard with *Culicoides*. Many of them have joined the team after the outbreak of  
bluetongue virus (BTV) in northern Europe in 2006, like myself, but some have  
45 a lifetime of experience with *Culicoides*. I particularly would like to mention  
here the morphologists Rudy Meiswinkel and Jean-Claude Delécolle, who have  
gathered incredible knowledge of the different species of this genus.

### 2.1.1 Taxonomy

50 Taxonomically, *Culicoides* is a genus in the family Ceratopogonidae, also known as biting midges, comprising 5,540 described small haematophagous flies [87]. The genus *Culicoides* comprise about 1,400 described species [105]. The Ceratopogonidae is placed in the suborder Nematocera which also comprises mosquitoes (Culicidae), sand flies (Psychododae: Phlebotominae) and black flies (Simuliidae), who are also vectors of numerous other diseases. Nematocera belongs to the order of two-wings, Diptera. 43 species of *Culicoides* are currently recorded in Denmark [103] of which some are potential vectors for bluetongue virus (BTV) and Schmallenberg virus (SBV). BTV and SBV have been isolated from wild-caught species of the Obsoletus group and the Pulicaris group [27, 110, 108, 42].

### 2.1.2 Vector species

The males of the Obsoletus group, comprising *C. chiopterus* (Meigen) *C. obsoletus* (Meigen) *C. scoticus* (Downes and Kettle) and *C. dewulfi* (Goetghebuer) in Denmark, are easy to recognize on their genitals. However, the females are not easily separated morphologically. The maxillary palps can be used for separating species, but this requires a substantial effort and time used on each specimen [97]. Thus, in studies with a large number of specimens, the wing pattern can be used as a shortcut to recognize species and species groups. The wing pattern is unique for many species, but it can vary to some extent, and on some specimens the pattern is very vague (Fig. 2.1). Therefore it is convenient in ecological studies to group closely related species in order to be able to identify a high number of specimens. The other species group suspected for transmission of BTV and SBV in northern Europe is the Pulicaris group. In this project this group comprises *C. pulicaris* s.str. (L.) and *C. punctatus* (Meigen), which are dominant on farms in Denmark (pers. obs.). These two species are relatively recognised on and separated by their wing pattern, although some specimens can show characteristics of both species (pers. obs.). In the present study the Pulicaris group and the Obsoletus group were used as the study units to minimize the time used on morphological identification. The species groups were identified following Campbell & Pelham-Clinton [22] and Glukhova [53]. Both species groups are commonly found on farms in northern Europe [4, 29].

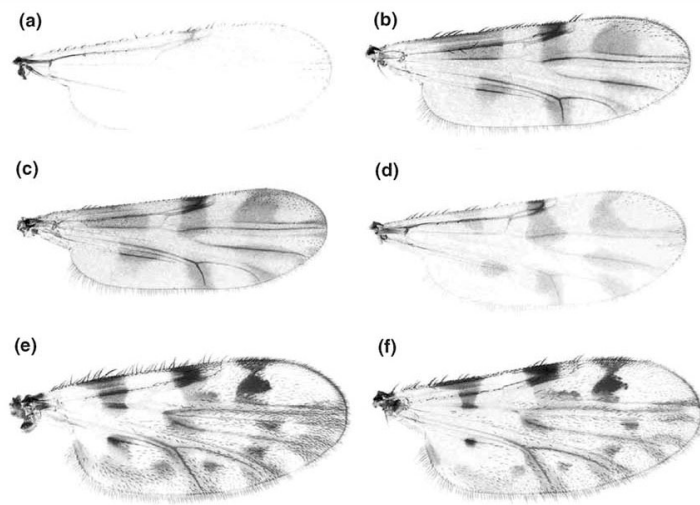


Figure 2.1: Wing patterns of the species included in the studies of this project: a) *C. chiopterus*, b) *C. obsoletus*, c) *C. scoticus*, d) *C. dewulfi*, e) *C. pulicaris*, f) *C. punctatus*. Reprinted from Carpenter, S. Wilson, A., Mellor, P.: *Culicoides* and the emergence of bluetongue virus in northern Europe. 2009, with permission from Elsevier.

### 2.1.3 Breeding sites

The breeding sites of the two species groups are generally not well investigated. Zimmer et al. [136] found *C. obsoletus* s.str., *C. dewulfi*, *C. chiopterus* and *C. scoticus* emerging from samples of maize silage residue from a farm in Belgium. Ninio et al. [99] found *C. obsoletus* s.str. hatching from manure collected outside farm buildings in France. Gonzalez et al. [56] found *C. obsoletus* s.str. in manure from farms and *C. scoticus* in rotting leaf litter. Surprisingly, only few studies have succeeded in finding the larval habitats of the Obsoletus group, despite they are one of the most dominant species groups in light traps on farms in northern Europe. The same is true for the Pulicaris group, which have been found breeding in water-logged areas on pasture, in the mud zone of ponds and marshes with organic material [96, 76, 56]. During the field studies in this project, breeding sites were sampled using emergence traps as shown in Fig. 3.2 (right) [76].

### 2.1.4 Behavior

One of the most important and puzzling aspects of *Culicoides* vectors is their behavior. They are too small for the human eye to follow in flight, which has given them the nickname 'no-see-ums' in North America, because they are almost invisible until they bite. Most species are crepuscular and therefore active at dusk and dawn but almost not in the middle of the night. The females suck blood from a variety of hosts, and males feed on nectar [87]. Different species have different host preferences [8, 80]. Downes [39] observed male swarms of several species, and even female swarms of some species, including *C. obsoletus*. He also mentions that female swarms are recorded in other genera within Ceratopogonidae.

*Culicoides* are commonly caught in light traps (Fig. 2.2). Like other insects, they fly towards light at night. In the light traps they are sucked down by a fan and into a collection beaker. The beaker can be filled with watery solutions for killing, or it can be empty for catching the specimens alive.



Figure 2.2: Two widely used trap types for *Culicoides*: The CDC mini trap type with a 4 W light tube hung in a heavy metal gallows on a field with sheep (left); and the Onderstepoort type with an 8 W light tube hung in a goat stable (right).

## 2.2 The outbreaks

### 2.2.1 Transmission in the vectors

Two vector-borne diseases have been transmitted by *Culicoides* in the last six  
115 years in northern Europe: bluetongue virus and Schmallenberg virus. When a  
vector bites an infected host, the virus in the blood meal will pass through the  
gut wall of the vector and start to replicate. The virus will spread throughout  
the vector, and at some point enter the salivary glands, ready to be injected in  
the veins of a new host. The period from when the blood meal is taken from  
120 an infected host, to when the virus has replicated and is ready for injection in  
another host, is called the extrinsic incubation period and lasts about 4-20 days  
dependent on e.g. temperature [105]. During this period, the vector will use  
the nutrition from the blood meal to produce eggs, then find a breeding site  
and lay up to 450 eggs [92]. Vector-borne diseases has not been observed to be  
125 transmitted transovarially [115]. The eggs hatch in 2-7 days, and the larvae will  
either overwinter or pupate after a period that can be as short as two weeks  
[92]. After laying the eggs, the vector will seek another host to bite for the next  
blood meal.



### 2.2.2 About bluetongue virus

*Culicoides* have not been of much interest in the past centuries in northern Europe. They have been a nuisance, but they did not cause more trouble than painful bites and sweet itch in horses [5]. Bluetongue virus (BTV) is a vector-borne disease that can cause fever, cyanotic tongue, salivation, swellings on the head, decreased milk production and increased morbidity and mortality in ruminants. There are currently found 25 serotypes of BTV, of which some are endemic in many parts of the world [36]. In the Mediterranean region, BTV is mainly transmitted by the subtropical vector, *C. imicola* (Kieffer) [33]. This vector species is distributed in Africa and in the southern parts of southern Europe [105]. In the 19<sup>th</sup> century there were sporadic outbreaks of BTV in southern Europe within the distribution of *C. imicola*. But since 1998, continuous waves of different strains of BTV have flushed into southern Europe from north Africa. [105]. In 2006, BTV took a new step into northern Europe.

### 2.2.3 Bluetongue virus in northern Europe

BTV serotype 8 emerged in northern Europe in the autumn of 2006, where it first appeared in The Netherlands, Belgium, Luxemburg, Germany and France [43]. It had only previously been recorded in Africa, Central America and Asia [43]. It soon became clear that new vectors were responsible for the spread of this emerging disease, as it was found outside the distribution of *C. imicola* [90]. At first it was speculated that the outbreak could die out with the vectors during the cold winter in this region. Unfortunately it survived the winter [134] and the outbreak continued the following years, reaching Norway and Sweden in 2009 [41]. The costs of the outbreaks have been costly for the farming industry. In the Netherlands alone, it was estimated that the net costs of the 2006 outbreak was 32.4 million Euro, and for the 2007 outbreak 164 to 175 million Euro [128]. A vaccine for BTV serotype 8 was released in 2008, and vaccination began in many countries in northern Europe [31, 122].

### 2.2.4 Schmallenberg virus in northern Europe

In November 2011, a new virus was discovered in Germany [70]. This new virus, named Schmallenberg virus (SBV) after the village where it was first found, belongs to the Simbu serogroup of the *Orthobunyavirus* genus comprising vector-borne diseases [11]. It infects ruminants and is most related to other viruses in Australasia, e.g. Shamonda virus [55]. SBV has mainly been found in sheep,

cattle and goats. The symptoms of SBV can be decreased milk production, late abortions, malformations of fetuses. In early 2012 it was confirmed that SBV was transmitted by *Culicoides* [108]. At the time of writing, SBV has been found as far north as in Finland [32]. The speed with which SBV has spread is faster than the BTV outbreak [47]. A possible reason for this is a higher prevalence of SBV in the vectors. Elbers et al. [42] found a prevalence of 0.56% for SBV in caught specimens of the *Obsoletus* complex, about ten times higher than found for BTV. There are still many uncovered aspects of SBV, and the main concern now is to estimate parameters for modeling the disease and work out a control strategy.

## 2.3 The tools

### 2.3.1 Models

In case of an outbreak, modeling the spread and impact of the disease is a cost-effective way to test different scenarios that are often impossible to test in real life. During the BTV outbreak in northern Europe, vaccination was rolled out as soon as a vaccine became available in early 2008 [135, 122]. However, vaccination is costly and an alternative approach is to model different scenarios in order to evaluate different control strategies. Several model frameworks have already been developed for BTV in Europe [60, 121, 66, 41, 58].

A common modeling approach is to estimate the vectorial capacity. The vectorial capacity is defined as the number of new infected hosts caused by one infected host per unit time (e.g. per day). By modeling the vectorial capacity it can be investigated e.g. when an outbreak can occur, where it can occur, how it will spread, how many animals will be infected and how the outbreak can be minimized or even stopped. A simple version of the vectorial capacity, denoted by  $C$ , adapted from Garrett-Jones [48] and Garret-Jones & Grab [49], is shown here:

$$C = m \cdot a^2 \cdot P^n \cdot \frac{1}{-\ln P} \quad (2.1)$$

where  $m$  is the number of vectors per host,  $a$  is the biting rate,  $P$  is the survival rate of the vectors and  $n$  is the extrinsic incubation period in the vectors.

### 2.3.2 Model parameters

The higher the vectorial capacity, the faster an epidemic can grow. The vector capacity can be multiplied with the infectious period in the host to describe  $R_0$ , which is the number of new infections that will occur from one host. Other models, such as the one proposed by Græsbøll et al. [58], use stochastic simulation to simulate an outbreak, and have the possibility to build in the complex behavior of hosts and vectors. However, all models need to have proper input in order to accurately predict the spread of disease. As is the case for BTV and SBV, the model frameworks rely, among other factors, on estimates regarding the vector behavior and abundance. As seen in equation 2.1, one of the most important parameters is the vector abundance. If only few vectors are present in an area, the number of vectors per host is low and thus the probability of disease spread will also be low, and vice versa. Thus the vector abundance is crucial for modeling vector-borne diseases.

Another important parameter for the vectorial capacity is the biting rate. This depends on the vector species and the host species, but also on other conditions like the weather, which has an impact on the activity of the vectors. The vector survival rate is also included, and largely uninvestigated for European vectors. We have successfully hatched wild collected specimens of *C. festivi-pennisi* in the lab and fed them with sugar solution where some specimens survived up to 29 days in captivity. But the vector survival rate will likely vary with factors such as temperature [17]. The incubation period within the vectors, which is the time interval from the infection of a vector to when it becomes capable of transmission to a new host, does also vary with temperature, because the virus replication rate is temperature dependent.

### 2.3.3 Vector dispersal

Another important parameter for the models is the dispersal of vectors. Many models use spread kernels to simulate local spread of the disease in addition to passive wind dispersal over long distances (e.g. [40, 67, 58]). As shown in Græsbøll et al. [58], the modeled disease outbreak is dependent on the distance between hosts. If this distance is small, vectors can transmit disease between hosts faster. However, in northern Europe, it has not before been investigated how the vectors are moving between different areas, and therefore the transmission between hosts is not empirically based. Therefore information is needed regarding the distance, direction, speed and the proportion of the population that disperse between farms. If, for instance, this local dispersal is not omnidirectional as suggested in Brenner et al. [20], it can be related to the wind

direction. If the flight behavior of *Culicoides* is similar to mosquitoes, they are likely to fly upwind in order to find a host, a behavior pattern known as anemotaxis [87]. This has not been investigated in northern European vectors before.

235 The present PhD project focuses on the patterns of vector abundance and dispersal in order to estimate parameters for models of vector-borne diseases like BTV and SBV.



# Outline of the thesis

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## 3.1 Aims and perspectives

The focus of this project is the spatio-temporal abundance and dispersal of *Culicoides*. This is investigated with regards to the potential vectors of BTV and SBV, in northern Europe comprising the Obsoletus group and the Pulicaris group [110, 108, 42]. The abundance and dispersal of these vectors is generally poorly investigated and thus results of the project can contribute to current models for the spread of vector-borne diseases.

There are four aims of this study, which are described below:

1. Investigation of the spatio-temporal abundance pattern of *Culicoides* vectors on a local scale.

This first part of the project is carried out to provide basic knowledge on the spatio-temporal abundance pattern of *Culicoides* vectors on a local scale. The abundance of vectors is expected to be higher in the vicinity of some significant spatial factors such as hosts and breeding sites, but this has not been investigated thoroughly before for northern European vectors. Likewise, temporal factors such as temperature and wind are likely to have an impact on the vector

activity. Therefore, relevant temporal factors are assessed for impact on the vector abundance. In this part of the project, repeated field experiments is a key element.

Monitoring programmes and sampling experiments for estimating parameters for models of disease outbreaks have previously been conducted without considering the local spatial and temporal variation, i.e. sampling with one trap for one night has been used to represent the vector abundance in a large area. This means that the distribution of vector abundance may be influenced heavily by undetermined spatial factors around farms. If we can identify any significant spatial factors for the vector abundance, it will be possible to adjust the modelled abundance estimates according to those factors. Thus there is a potential for greatly improving the precision of epidemiological models.

## 2. Optimized strategy for sampling of vectors.

After the first study has been conducted, addressing the spatial abundance pattern, the next step is to find out how to adjust the sampling procedure with regards to the spatial and temporal variation. By taking significant spatial and temporal factors into account, it will be possible to standardize sampling programmes with regards to those factors. The goal is to develop practical guidelines for future sampling programmes.

Surveillance programmes and sampling experiments are costly, both in terms of the hours used for catching and, perhaps even more important, the hours used for sorting the catches. Thus the sampling design should be cost-effective. For instance, it is relevant to find out if a significantly better estimate is obtained by sampling more than one night in an area. Thus it will be possible to design future sampling studies with maximal outcomes (in terms of area covered and precision of estimates) for minimal costs. Furthermore, it will be possible to determine the number of traps needed to estimate the vector abundance in an area with high certainty.

## 3. Estimation of the range of attraction for light traps catching *Culicoides* vectors.

It has not previously been investigated how far the range of attraction for the battery-driven CDC 4 W light trap is. This trap type is used in many studies for sampling *Culicoides*. In order to determine the optimal location for traps when sampling near breeding sites, hosts, etc., it is important to know the range from which vectors are actually attracted to the trap. The aim is therefore to



Figure 3.1: Left: A trap placed sub-optimally for sampling vectors near host animals. Right: The optimal trap placement for catching vectors near host animals.

estimate the range of attraction for *Culicoides* for this trap type with regards to the potential vectors of BTV in northern Europe.

The result is useful for studies that aim to cover a specific area with traps, or studies where numerous traps are set up in an area to investigate the spatial abundance on a local scale like in the first part of the present PhD project. Often, in sampling programmes, a trap is placed sub-optimally because it must be placed out of reach of agricultural machinery and host animals. Thus it is important to know the range of attraction from any suboptimal position.

4. Quantifying the dispersal of *Culicoides* vectors between farms.

Only very few experiments have addressed the dispersal of *Culicoides*, and never before in Europe. The aim in this part of the project is therefore to estimate the dispersal of vectors between farms. The key parameters describing the dispersal are: The distance at which the vectors disperse; the speed at which they disperse; the dispersal direction; and the proportion of vectors that actually disperse between farms. These parameters are investigated for vectors on farms in Denmark.

At the moment, the dispersal of vectors in BTV models rely on expert opinions and a few estimates from field studies in North America. However, the dispersal is a key parameter in epidemiological models for vector-borne diseases and therefore should be thoroughly determined in order to produce accurate model results. The outcomes of this study are directly applicable in e.g. BTV models.





Figure 3.2: From the field study in 2009. Left: The shepherd leads the sheep into an enclosure in the end of the field in the evening. Right: Emergence samples are set up for hatching adult *Culicoides* from soil and mud samples in a stable building near the study site.

## 3.2 Field studies

This PhD project is based on field work from four locations in Denmark during three field seasons: 2009, 2010 and 2011. Data from the 2011 study are yet unpublished, but described in this thesis. All field work was carried out by the author, with help from local farmers and shepherds. All study sites were placed within the region of Stevns, which is characterised by being very flat and with a large proportion of agricultural land. The traps used for field studies were CDC 4 W 1212 Mini UV-light traps ([www.johnwhock.com](http://www.johnwhock.com)) that run on batteries and have a photoswitch to automatically turn on at dusk and off at dawn. Special gallows of welded iron were constructed to be able to hang up the traps on a field with sheep. The sheep would rub their sides against the gallows during the day, and therefore they had to be solid.

In 2009, the study site was a field (approx. 250 m wide and 750 m long) with 260 sheep in Vallø (see Appendix A and B). *Culicoides* were collected here using 45 traps on the field from July to September. The traps were put up in a grid formation, separated by 50 m. Thus the whole field was sampled for vectors at night. This field study was conducted to investigate the spatial and temporal variation on a local scale. Sheep had access to the field during the day but were moved to an enclosure during the night when the traps were turned on.

In 2010, the study site comprised a dairy cattle farm with 700 livestock near Holtug in Denmark; a pig farm with 1700 pigs; and a beef cattle farm with 20 angus livestock. The two latter were located 1750 m and 2000 m west of the

dairy cattle farm, respectively (see Appendix D). The focus of the field study was to conduct mark-release-recapture experiments, and to sample the spatio-temporal abundance of vectors on a slightly larger scale than the previous year. Traps were hung up around the field in four directions, as far away as 2 km.

In 2011, two study sites were used: The first study site was a farm with 50 dairy cattle near Lille Heddinge in Denmark (geographical coordinates: N55.2818, E12.3907). In a radius of 1 km to the study farm, 12 traps were used to sample the abundance on 12 catch nights from August 18<sup>th</sup> to August 29<sup>th</sup>. These data were collected to explore the spatio-temporal abundance around the farm (see chapter 4). The second study site was a farm near Klippinge with 70 livestock, where different configurations of trap setups were used in modeling the range of attraction for light traps (see Appendix C). The traps were emptied every hour during the field experiments and taken down during the day to let the cattle graze in the area.



# Spatio-temporal abundance of vectors

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## 4.1 Introduction

The vector abundance is an important parameter when modeling the spread of *Culicoides*-borne diseases (e.g. [66, 121, 61, 88]). When sampling for vectors on a farm, a field or even in an urban area, the location of the light trap is important. Is it better to place it in one end or the other of a stable? Will two different trap locations on a field or a farm catch similar numbers of vectors or are there areas with higher or lower abundance? Is it better to place a trap right between host animals and a breeding site? Does it matter if the trap is placed in the near vicinity of the host animals or do the vectors fly around, eventually getting caught anyway? Are there areas where the abundance is higher than others, and can we link this higher abundance to objects such as hosts or breeding sites? Is it then possible to predict the abundance in other areas? Is the spatial abundance pattern similar between different vector groups? Do temporal factors have an impact on the vector abundance? In this chapter we investigate the vector abundance on a local scale. We explore factors determining local vector abundance, and quantify the abundance spatially and temporally. Thus we start by asking a key question:

- What does the spatial abundance of vectors look like on a local scale?

## 4.2 Abundance related to hosts

The presence of hosts is an important factor for vector abundance [46]. Therefore we will first have a look at how vectors were distributed around hosts in the field studies of this project. This is to get a general impression of how vectors are distributed around farms on a local scale.

In the data from the field study in 2009, the model results show that the abundance is higher near the sheep and decreases until 300-400 m away (see Fig. 3 in Appendix A). In the 2010 field study, the abundance of vectors was measured on a larger scale (see Appendix D). The effect of the distance to the host animals was similar, but the lowest abundance was here found at a distance of 800-1000 m (Fig. 4.1 left).

In the 2011 data, an abundance pattern very similar to the pattern in the 2009 data is seen: A higher abundance is present near the host animals, and then comes a dip in the abundance at 300 m from the hosts, and then the abundance increases again up to 500 m (Fig. 4.1). This pattern could be caused by a depletion effect surrounding the host animals. Another explanation could be that other factors further away causes the abundance to increase, but this is untested in this study.

A regression line with a quadratic term for the distance to hosts was fitted to the data to show the general trend (Fig. 4.1). The data presented here were not investigated with respect to spatial autocorrelation and thus the true abundance pattern can be different. However, as seen in table 3 in Appendix A, the ordinary regression models approximated the CAR model estimates regarding the effect of the distance to host animals, so the pattern in Fig. 4.1 might reflect reality well.

Lühken and Kiel [85] conducted a similar study in 2009 where 13 traps were hung in distances of 0 to 200 m from a farm with 220 livestock. They found a pattern of decreasing abundance of females of the *Obsoletus* group and the *Pulicaris* group with increasing distance to the farm up to 200 m. They did not

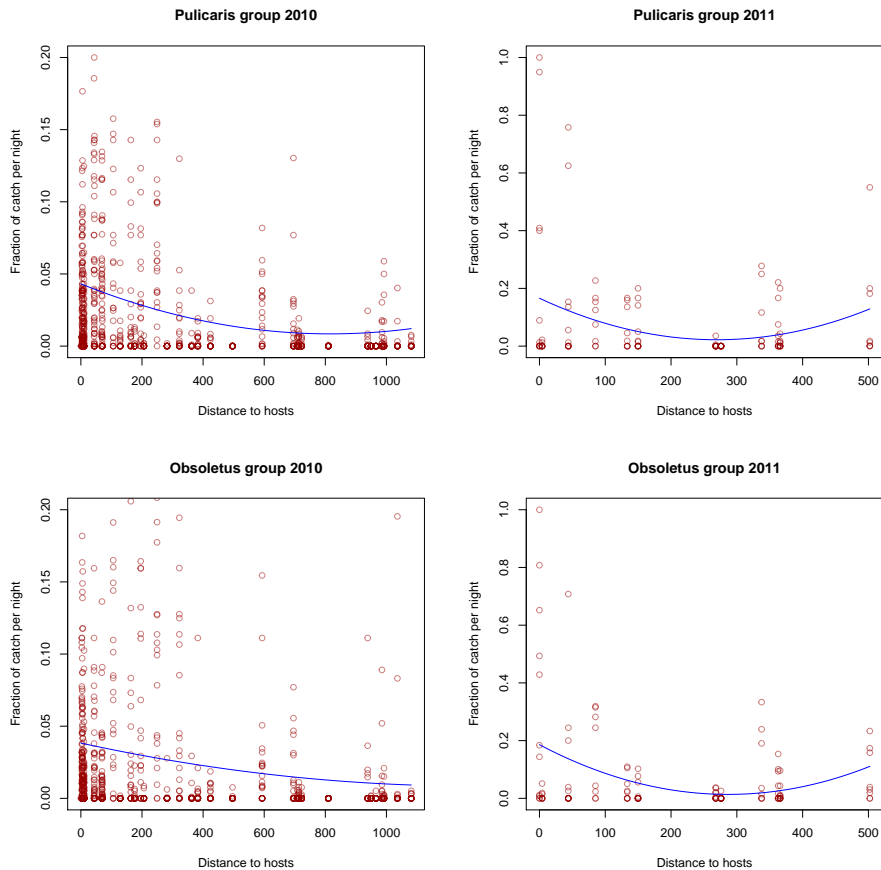


Figure 4.1: In 2010 and 2011, the abundance of the Obsoletus group and the Pulicaris group was estimated using light traps placed around two farms. The vertical axes show the fraction of catch per night. The 2010 study is described in Appendix D. Fractions above 0.2 are cut off in the 2010 dataset to focus on the linear regression line. In the 2010 data the distance to host animals is calculated to the nearest host location (cattle, pigs or the small angus herd described in Appendix D).

find an increase in the abundance further away from the farm, perhaps due to the investigated range of distance to hosts. Rigot et al. [112] also conducted a study in 2008 and 2009 where it was found that the total catch of female *Culicoides* decreased up to 250 m away from the five farms investigated.

The findings in the 2009 study, the 2010 study and the 2011 study presented here, and those of Lühken and Kiel [85] and Rigot et al. [112] show the same pattern: An increasing vector abundance with decreasing distance to the host animals, within different ranges. However, the patterns are not entirely the same. The distance from host animals where the lowest abundance is found differs between the studies. This can be a result of differences in attraction based on the number of host animals, or differences in the surroundings in the investigated areas. If an area is hostile to the vectors, they would probably be more concentrated around the hosts.

## 4.3 Spatial modeling

### 4.3.1 Spatial autocorrelation

The results showed above using ordinary regression modeling can be an indicator for the spatial pattern related to different attractors such as hosts. However, they do not take spatial autocorrelation into account. Spatial autocorrelation means that spatial measurements can be dependent on each other because of their position. An assumption for regression modeling is that the observations are independent of each other. Measurements that are closer to each other can be more similar because of an unknown underlying pattern. This pattern can for instance be caused by spatial clusters. In the analysis of the 2009 study (described in Appendix A) the spatial autocorrelation was taken into account using the block design formulated by Besag [14]. In this model design, an observation is regarded as independent of all other observations than its neighbors in a grid (see Appendix A). The dependency between (first order) neighbors is modeled as the spatial autocorrelation factor,  $\rho$ , which describes the general dependency of neighboring observations. The resulting model is a conditional autocorrelation (CAR) model. In the next sections, the findings and implications of CAR models for the two species groups are further discussed.

### 4.3.2 Spatial covariates

The 2009 study was conducted to have a closer look on the abundance of vectors on a local scale (described in Appendix A). We hypothesized that the abundance was higher in the vicinity of host animals. Thus, one of the aims was to quantify the impact of the distance between host animals and light traps on the vector abundance. In addition to quantifying the impact of host animals, other spatial factors were also tested in this project. The aim was to determine if there were spatial factors in rural areas that caused higher abundance of vectors. If any spatial factors could be identified as causing higher abundance, it would have an impact on the transmission of disease. Potentially, areas with low risk of transmission could be identified. Perhaps more importantly, trap catches in other studies could potentially be adjusted with regards to any significant spatial factors in order to standardize the estimates of vector abundance. As described in Appendix A, no spatial factors other than hosts were identified as significant for the abundance of vectors.

This pattern has also been found in other studies [85, 111]. In this study we were able to quantify the abundance near the sheep compared to further away, and found that the *Obsoletus* group abundance was twice as high as in the middle of the field. For the *Pulicaris* group the abundance was 50% higher near the sheep than in the middle of the field. The model in this study included a quadratic term and the abundance estimate is higher further away from the host animals than in the middle of the field. Since we tested this model type against a log type model, this higher abundance away from the sheep is most likely not an artefact. In the southern end of the field there was a forest area, which could cause a higher abundance of vectors. However, the higher abundance could also be explained by depletion in the abundance of vectors in the vicinity of host animals. Unfortunately, we were not able to test if the higher abundance was correlated with the forest since the sheep and the forest were placed opposed to each other on the field. If we had placed the sheep in the southern part of the field on half of the catch nights, we would have been able to separate the effect of sheep from the forest.

### 4.3.3 Temporal covariates

The temporal variation in vector abundance was also addressed. The study period was from July 20<sup>th</sup> to September 4<sup>th</sup>, and comprised 16 catch nights. Within such a long period, fluctuations in the abundance level are expected.



A number of temporal covariates were therefore also tested in this study to determine their impact on the abundance. Five factors were found significant: Precipitation, turbulence, humidity, temperature and wind speed (Appendix A). As was expected, there was a positive correlation between humidity and vector abundance. What was also expected was a negative correlation with precipitation and turbulence. Temperature and wind speed were included in this model with a quadratic term, and the former showed a peak in abundance at 16 °C. This correlation is probably species specific, and most likely the activity of sub-tropical vectors like *C. imicola* will peak at higher temperatures. This effect can also be linked to the study area, and thus the activity of the same vector species may peak at higher temperatures in the UK for instance. The same could be true with regards to seasonality, so the peak activity may change with generations of vectors hatching through the season. The wind speed showed a positive correlation with abundance in the measured interval (0-5 m/s). Logically, small insects cannot fly at really high wind speeds, and therefore it is expected that the correlation between wind speed and vector abundance will decrease again at some point above 5 m/s.

#### 4.3.4 Spatial clusters

The modeling framework used in this study was a conditional autoregressive (CAR) model framework that was able to take the spatial autocorrelation (i.e. spatial clustering) into account. Thus the spatial autocorrelation was removed from the dataset in the modeling procedure. Then the spatial and temporal factors included in the models were tested and their impact estimated. The models were compared to ordinary regression models that did not remove the spatial autocorrelation and thus yielded incorrect estimates of the significant factors (table 3 in Appendix A). As mentioned above, the distance to hosts does not differ much between the two model types. In the CAR model we were able to subtract the correct impact of the correct significant factors from the data. When subtracting the CAR model effects from the original data, the clusters of abundance were revealed. These clusters showed a dynamic pattern where they moved to a new place for each sampling night (Fig. 4 and 5 in Appendix A).

Since we used a grid design to put up the traps, we could use Besag's block design to describe the spatial autocorrelation for each trap [14]. In the used modeling procedure, a general autocorrelation,  $\rho$  was estimated. However, the degree of spatial dependence may differ between locations on the field and between catch nights, which is not possible to distinguish in this type of model.

## 4.4 Challenges

One of the main aims of this study was to investigate the impact of host animals on the vector abundance. We showed that the host animals did have a significant impact, and for the *Pulicaris* group we also saw an impact of pairs of sheep placed in a transect on the field (see Appendix A). In the CAR models the vector abundance is increased near the sheep. The closest trap position to the sheep is 38 m, and thus we are not able to estimate the vector abundance right next to the sheep, which is a shortcoming of the study design. A limitation of this study is that only one field was investigated. If more fields were used in the study it would have been possible to speculate if the found pattern was general on fields in Denmark. Moreover, we cannot extrapolate the findings in this study to other numbers of host animals. In order to do so, we could have removed half of the sheep on half of the catch nights, and then we would be able to estimate if there were more vectors present on the field (attracted to the sheep) on nights with more sheep. In 2009, Garcia-Saenz et al. [46] conducted a study where different numbers of sheep were placed under a light trap for attracting *Culicoides*. They found a significant increase in the number of *Culicoides* using zero to three sheep, but no increase when using six sheep. This indicates that the attraction effect of each host animal is more pronounced when small numbers of hosts are present. Another limitation of the experimental setup in the present study is that we are not able to say anything about vector abundance related to other host animals such as cows. It would be interesting to investigate if there is a difference in attraction between cattle and sheep, which could be done by changing the host animals during the study. Perhaps a cow attracts twice as many vectors than a sheep because of its size, emitting more exhaustion gasses and body odours.

Mayo et al. [88] recently showed that the infection rate of BTV in Californian vectors were higher in specimens collected with an aspirator directly on cattle than specimens collected in light traps nearby. This underlines that light traps are not the same as host animals and thus trap catches may not represent the number and life-stages of vectors that are attracted to hosts, but merely those who are attracted to light traps. This is a serious weakness and can potentially skew the results of all studies using light traps to estimate the vectors with regards to hosts [26].

Other hosts, like Roe deer, may be present at night when catching *Culicoides*. We have seen that even a pair of sheep can have a significant impact on the abundance of the *Pulicaris* group. If roe deer entered the field at night, they could be responsible for creating a cluster of higher vector abundance in their vicinity. However, there were no sources of food other than grasses and shrubs present on the field that could attract roe deer. Moreover, roe deer do not like

to feed on grass with sheep dung. No other hosts were seen on the field upon inspection of the setup in the evening.

## 4.5 Conclusions

555 In this study we found the following temporal factors significant for the vector  
abundance on the field: Precipitation, turbulence, humidity, temperature and  
wind speed. We also found that the distance to hosts was significant for vectors,  
and the abundance of the *Pulicaris* group was also positively correlated with  
560 small groups of hosts. We found that clusters with higher vector abundance  
were present on the field, and that they moved between catch nights. We showed  
that there was no significant temporal autocorrelation in the data, but there was  
a significant spatial autocorrelation present. The resulting models for vector  
abundance were found valid, but their precision was low due to the unpredictable  
dynamic abundance pattern.

# Optimized sampling of vectors

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## 5.1 Introduction

In the previous chapter we have seen how the abundance of vectors can vary in space and time. This dynamic pattern makes it difficult to obtain precise estimates of the abundance in monitoring studies. However, the number of vectors is one of the most critical parameters when modeling the spread of vector-borne diseases [121, 58]. So how do we take the current knowledge of dynamic spatial clustering into account in sampling studies? What is the optimal strategy for sampling of vectors? Here we take a closer look at this and explore the properties of the data from the 2009 study (described in Appendix B).

In this study we quantified the sampling variation on each catch night, in order to quantify the increase in precision of the estimated abundance by using a higher number of traps. We also tested if the estimated abundance was more precise if two or more traps were separated by a minimum distance on the field, in order to reduce noise from the spatial clusters. In a third analysis the risk of falsely detecting absence of vectors on a field was calculated. And lastly, the number of traps needed to reach 90% and 95% certainty of detecting presence of vectors on the field was calculated.



Figure 5.1: The time used for sorting the *Culicoides* can be costly in sampling programmes. Left photo: DTU/Kaare Smith.

## 5.2 Spatial sampling

We found that the estimates of mean vector abundance was improved by including more traps, and using few traps tended to underestimate the mean vector abundance. The reduction in variation in the estimated mean abundance from adding one more trap to the study was more pronounced when few traps were used. When six traps were included, the variation in the estimated mean vector abundance was reduced by 50%.

Theoretically, the spatial clustering pattern will make it difficult to obtain a precise estimate of abundance because some traps will hit a hotspot by incidence, and some will not. Therefore it could theoretically be better to separate two or more traps spatially within the study area so the probability of sampling both places would be higher. However this was not the case. We found no indication of a more precise mean estimate when separating the traps.

## 5.3 Temporal sampling

In the 2009 data a considerable amount of temporal variation was found. When sampling for *Culicoides* in the field it would be convenient to know the effect of sampling two or more nights in the same place, rather than only one. This might increase the cost of a field study, but it could also increase the quality

of the outcome. In this study we explored the dataset from 2009 in order to describe the increase in precision by including more than one catch night (described in Appendix B). We found that by including two nights instead of one, the number of traps needed to reach 95% certainty to detect presence of a vector on a field, dropped from 25 to 7. Thus the optimal sampling strategy is not to sample only one night per location as in some studies [89, 98], but to include more than one night from each location, as in other previous studies in northern Europe [37, 69, 79]. However, if it is possible to avoid sampling on nights with low vector abundance (or activity), the 95% certainty of detection can be reached with only 3 traps when sampling on one night. This suggests that field sampling should be, if possible, optimized according to temporal factors based on meteorological forecasts such as those found in the CAR models (Appendix A).

## 5.4 Challenges

The field study in 2009 was originally designed for the analysis described in Appendix A, and not for the present analysis. However, we decided to use the data to investigate the questions described here, in order to evaluate the practical implications of the variation in abundance. The problem of using a single field study to investigate the spatial and temporal properties of different sampling designs is that the results cannot be representative for other areas. Thus the observed pattern can be different in other areas. Presence/absence studies are, of course, greatly influenced by the level of abundance, and therefore it is not straightforward to apply the results of the study presented here to other situations. But the present study can be used to give an idea of the certainty of such sampling.

The methods used in this study explores the spatial abundance pattern in the dataset, including some assumptions. Most important is the competition between traps on the field. We here define competition as the impact on a trap catch when adding one or more traps to the area. The trap catch in one trap will thus decrease because vectors that would be caught in the first trap are caught in another. The result is a degree of depletion of vectors on the field. Therefore traps in the periphery will, in case of substantial depletion, catch higher numbers of vectors, migrating in from the surroundings. However, we did not observe such a pattern and therefore we consider the competition between traps in the present study of minor importance.

## 5.5 Conclusions

In this study we found that there was an increased precision in the estimated abundance when using more traps on the field. We found no effect of separating the traps with a minimum distance when using more than one trap on the field. We explored the risk of falsely detecting absence of vectors on the field when using one or more traps for sampling. If nights with low vector activity can be avoided, the estimate will be highly improved. It was also shown that spreading catches over more than one catch night greatly reduced the risk of falsely detecting absence of vectors on the field.

In this field setup it was hypothesized that the range of attractions of a trap was less than 25 m, because otherwise they would overlap and thus may complicate the setup. In the next chapter we conduct a study to test this by estimating the range of attraction for this trap type.

## CHAPTER 6

# The range of attraction of light traps

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### 6.1 Introduction

In the previous chapters we have explored the abundance pattern of vectors using light trap sampling. In this chapter we will estimate the range of attraction of the CDC light traps and thus test if the assumption of no overlap between traps in the 2009 resampling study was fulfilled (Appendix B). We conducted  
a study where traps were hung in transects with different distances in between,  
thus competing for *Culicoides* with each other. This study design initiated a  
larger study where some of the basic mechanisms in *Culicoides* behavior were  
examined.

When sampling with more than one trap on the same field, three mechanisms  
are relevant to take into account: Firstly, the spatial autocorrelation described  
in Chapter 4 is a concern. Secondly, the competition between traps has an  
influence on the numbers caught in each trap because all traps in the study area  
will compete with each other (see Chapter 5). Thirdly, the range of attraction  
of the light traps will have an impact on the number of vectors caught. This  
third mechanism is the focus of this chapter. It is here defined as the range from  
which the vectors can perceive the light from the light trap and show directed



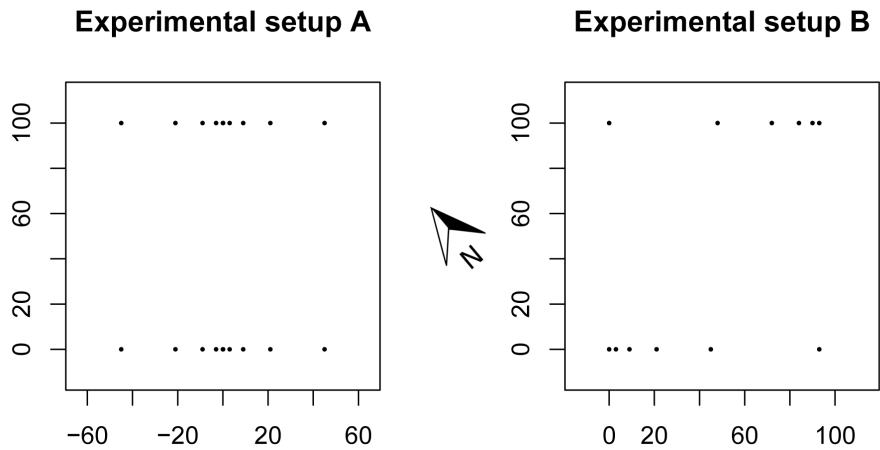


Figure 6.1: The setups used in the study. The two trap configurations on the field are shown. This figure is also shown in in Appendix C.

movement towards it, also known as phototaxis.

The experimental setups and methods used in this study are further described in Appendix C.

## 6.2 Field sampling

The focus of this field study in 2011 was to estimate the range of attraction for the light traps, and the intended analysis was to use a model framework similar to the one used by Rigot et al. [111]. In this framework, the range of attraction was estimated by assuming a circular range of attraction of the traps. The range of attraction was then defined as the range where the proportional trap catches were best approximated to field data.

In the present study, Setup A was conducted first: A transect of traps was set up where the traps were placed closer and closer to each other towards the middle (Fig. 6.1 (left)). When some of the transect catches were counted during the study period, an unexpected pattern was discovered: The traps in the middle of the transects that were placed close to each other, showed a higher catch than the outer traps in the transect that were placed with more spacing (see fig. 5

(left) in Appendix C). At that point it was clear that the attraction mechanism was more complex than previously assumed. When emptying the traps at night, it was easier to recognize traps hanging close together from a distance, than it was to recognize single traps (pers. obs.). This synergistic effect could also reflect how the vectors perceive the light.

After this, the field experiment with Setup B was conducted, allowing for a larger range of attraction before overlapping with a more distant trap in one end of the transect. Thus we had two field setups for fitting models.

## 6.3 Models

We used three different models to test three different hypotheses about how vectors perceive and are attracted to light traps. They are described in detail in Appendix C. The model of best fit was a model where vectors evaluate light sources in the horizon and fly towards the direction with strongest illumination.

The model of best fit estimated the range of attraction for the vectors to be 15.25 (95% C.I.: 12.7-18.3) m. This model showed that the range of attraction for two light traps in the same position is extended so that the covered area is twice the size as for one trap.

The area covered by a trap is dependent on the time used for sampling. Thus, when a trap is sampling for longer time, more specimens will come across the range of attraction. However, we modeled the range of attraction based on relative trap catches, thus excluding the factor of time. Therefore we have estimated the range of attraction for the light traps without regards to the time used for sampling.

## 6.4 Challenges

The light traps used in the present project have some limitations. First of all, the attraction to light traps must be different from the attraction to hosts because the attraction clue is different. Host attraction includes the attraction to scent or odor, which is overtaken by visual stimuli at some distance from the host.



Figure 6.2: From the field study in 2011. Left: Traps are hung up in a transect. Right: Traps are turned on after dusk, attracting vectors.

The actual aim of most studies using light traps is to estimate the number of vectors related to hosts and not light traps. The traps are not built to mimic a host, but simply exploits that insects are attracted to light.

The light traps are not truly reflecting the number of vectors biting hosts, and underestimate the number of vectors present [52, 88]. As described in Carpenter et al. [26], the light traps attract different species differently. This might also be true for life-stages of the vectors. Thus, results obtained from sampling light traps are not always directly applicable to the behavior pattern regarding hosts. This bias have does also impact the other studies in this project: We cannot be certain that we have estimated the underlying clustering pattern of abundance correctly because of the bias introduced by using the light traps for sampling. However, light trapping is a cost-effective method to sample vectors and thus it is an important tool for investigating vectors.

## 6.5 Conclusions

We found that the range of attraction for the CDC 4 W trap was 15.25 (12.7-18.3) m. We also found that the vectors can evaluate the light at a distance and fly towards the direction where they perceive the strongest illumination. Lastly, we found that traps with overlapping catch areas have extended ranges of attraction because of a synergistic effect of the light traps.

In the field study in 2009 (Appendix A and B), the light traps were hung up in a grid on the field with 50 m distance. This distance was hypothesized to be optimal for thorough sampling of the area without having overlapping ranges of attraction of the light traps. From the present study we can conclude that this

hypothesis was fulfilled.



# Dispersal of vectors

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## 7.1 Introduction

In the previous chapters we have explored the abundance pattern of the vectors, how to optimize sampling on a field, and how they are attracted towards light traps. These are some of the key parameters in e.g. BTV models. To estimate the spread of disease using simulation modeling, one important factor is still  
uninvestigated:

- How do vectors spread virus between farms?

We need to find out how the vectors move around in the landscape, especially between farms, in order to quantify the spread of disease [127]. More specifically, the dispersal speed, dispersal direction, dispersal distance and the proportion  
of vectors that disperse will be investigated.

It is possible to track individual insects using radiotransmitters. However, the smallest radiotransmitter weighs much more than a single specimen of *Culicoides* ([www.holohil.com](http://www.holohil.com)). Radio Frequency Identification (RFID) tags, which are frequently used in ID and payment cards, may become an option in the future,

but the cost of marking enough specimens to be able to recapture them in other places would probably be enormous. So until new technology is developed, dispersal studies of small insects are left to classic mark and recapture techniques, where specimens are marked, released and later recaptured. It is then possible to follow individual specimens in space and time. For such small insects as *Culicoides*, the only feasible marking method is with very small particles. The procedure is simple: A large number of specimens are marked, released, and then hopefully some of them will be recaptured later. One of the problems of marking studies of *Culicoides* is that the population size seems to be gigantic, and thus the probability of recapture is very little.

## 7.2 Marking methods

Previous marking studies of *Culicoides* have mostly used dyes for marking [83, 20, 82], but Holbrook et al. [71] used rubidium for marking *C. variipennis* (syn. *C. sonorensis*) and subsequent spectrophotometry for detecting the marked specimens. This technique involved marking the third instar larvae in plates before hatching. This is not possible with European vector species because they cannot be reared in captivity. Only two species of *Culicoides* are reared under lab facilities; *C. variipennis* (syn. *C. sonorensis*) as mentioned above and *C. nubeculosus*. In preliminary lab studies in the present project we succeeded in marking individual wild-caught specimens by feeding them a sugar solution containing dye. However, most *Culicoides* specimens did not feed on the sugar solution, perhaps due to stress in captivity, and therefore this technique was abandoned (Fig. 7.1).

In the present study we chose to develop a new marking method using fluorescein isothiocyanate (FITC), and an ELISA plate scanner for detection of marked specimens. This method is similar to the method used by Lilie et al. and Brenner et al. [83, 20, 82], but the marking agent is different and the detection technique is not by eye but in an objective scanning procedure.

Mark-release-recapture (MRR) studies are used worldwide on a range of animals to estimate various properties of different study organisms. The population size can be estimated from MRR studies as well as other important population parameters [119].



Figure 7.1: Preliminary marking studies in 2009: An initial marking method was tested on specimens in the field: A sugar solution with green dye was made available for insects too feed on. Unfortunately, *Culicoides* did not feed on the solution as much as other insects.



### 7.3 Field study

In the present field study *Culicoides* were marked and released at the study farm, and subsequently caught in traps in the surrounding area (described in detail in Appendix D). We recaptured two specimens of the *Obsoletus* group and 28 specimens of the *Pulicaris* group. The two *Obsoletus* group specimens were caught at the same location where they were released. Eight (29%) of the recaptured *Pulicaris* group specimens were caught at a pig farm 1750 m upwind from the release point within two nights from the time of release.

### 7.4 Challenges

Contamination is a threat to mark-release-recapture (MRR) studies of small insects. Because very small amounts of marking agent should be detectable, the method is highly sensitive to accidental contamination. Therefore, in the present study, all marking gear was kept in separate closed containers.

In order to recapture a proper number of specimens, a higher number of marked specimens than in the present study should be released. As suggested in the present study (Appendix D), supplementing the marked and released specimens with more vectors caught in other areas could be a solution. However, it is not investigated if the vectors show the same behavior when moved to another location.

### 7.5 Conclusions

In the present study we found that the vectors exhibit directional local flight, where about one-third of the *Pulicaris* group dispersed upwind. Marquardt et al. [87] speculated that the range where mosquitoes show host-seeking behavior is 20-35 m downwind from the host. This assumption implicates that flight is random when more than 35 m away from a host. The results from the present study indicates host-seeking behavior right from the release point to the recapture point because almost one third of all specimens were found in the same direction. A few specimens were caught in other directions, indicating that random flight, or flight related to other factors than hosts, may occur.

The present study supports the findings by Sedda et al. [118] where a model



Figure 7.2: From the field study in 2010. Left: Inside the stable with 700 cattle. Right: Four traps are hung up on the stable wall to catch vectors for marking.

including local upwind flight of the vectors was able to fit data from the 2006  
825 outbreak of BTV more precisely than without upwind flight. The technique  
used in this study is useful for marking *Culicoides* vectors in the field. However,  
more studies will have to be conducted before a dispersal kernel for modeling  
the spread of vectors can be derived from the data.

830 Since 97% of cattle farms in Denmark lie within 1,600 m distance to each other  
(Kaare Græsbøll pers. com.), this study shows that vectors are capable of  
spreading diseases rapidly between most Danish farms. This may explain the  
rapid progression of BTV and SBV in northern Europe seen in the last decade.



# Conclusions and future research

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## 8.1 Conclusions

This PhD study addressed relevant questions for studies of *Culicoides* vectors in northern Europe. They are further described in Appendix A-D. The main findings were:

### 1. Investigation of the spatio-temporal abundance pattern of *Culicoides* vectors on a local scale.

- The distance to hosts is an important spatial factor for the abundance of vectors.
- Several temporal factors are important for the abundance (and activity) of *Culicoides* vectors: Temperature, humidity, precipitation, turbulence and wind speed were important for both investigated vector groups.
- The variation in the predicted parameter estimates of the model was considerable: 20.4% to 304.8%.
- There was no significant temporal autocorrelation present in the data.

- Significant spatial autocorrelation was present in the data.
- An unexpected pattern was found; clusters of higher female vector abundance were found moving around on the field between catch nights. This pattern was not explained by any known factors but could be caused by swarming behavior.

## 2. Optimizing strategy for sampling vectors.

- We found no increased precision in the simulated abundance estimates when increasing the distance between traps on the field.
- The temporal variation in vector abundance on a field introduces great noise into sampling experiments. When sampling for presence/absence of vectors, the number of traps necessary to obtain a reliable estimate is very dependent on this temporal variation.
- Sampling on two nights rather than one is a cost-effective way to reach a high certainty of detecting the presence of vectors on a field.

## 3. Estimation of the range of attraction for light traps catching *Culicoides* vectors.

- The range of attraction perpendicular to the light tubes in the light CDC 4 W traps was estimated to be 15.25 m.
- The area covered by each trap was best estimated when simulating the anisotropic light emitted by the light tubes. Thus the direction of the trap is important for the sampled area.
- When traps are placed closer together than their range of attraction, their shared range of attraction is extended. Two traps in the same location can thus sample an area twice as large as one trap.
- The attraction mechanism for the *Culicoides* vectors is more complex than anticipated. Thus they are able to evaluate light sources in the horizon at a distance and fly towards the highest illumination.

## 4. Quantifying the dispersal of *Culicoides* vectors between farms.

- A new mark-release-recapture technique was developed to estimate the proportion of dispersing *Culicoides* vectors between farms. FITC was found useful for this purpose.

- Two nights after release, 29% of the recaptured specimens were caught 1750 m from the release point.
- The dispersed specimens were caught upwind of the release point.

## 8.2 Further research

There is still much more to investigate in this puzzling group of vectors. For instance, very little is known about their resting sites [24]. If the vectors rest on a tree stem pointing south, the temperature will inevitably be much higher, and thus also the replication rate of virus, than if they rest in leaf litter in a shaded forest. Another interesting issue is that now that we have estimated the range of attraction for the CDC light trap, what is the attraction range of a cow? Or 700 cows as in the field experiment in 2010 in this project? Or 1700 pigs? Different species have different preferences of hosts [80], which means that there is a lot of work to be done before a disease spread model for all vector species and hosts can be created.

In order to obtain the parameters for the models, it can be necessary to make some technological shortcuts. The dream scenario for a field entomologist is a scanner that can sweep across a field and detect the species, position and direction of all small flying objects. Such a tool is not available at the moment, but it could be in the near future. A promising method of detection at a distance is LIDAR (light detection and ranging), which uses reflected light signals in much the same way as RADAR, but on a very fine scale. Preliminary studies at Lund University in Sweden have been conducted on damselflies using marked and unmarked specimens, and it was possible to discriminate between species and even sex [59]. This system can possibly be applied to detect the species and sex of small flying insect such as mosquitoes and biting midges within a couple of kilometers range (Brydegaard pers. comm.). At the time of writing, Mikkel Brydegaard, one of the leading scientists in this field, is building a new mobile unit for LIDAR studies in the field, called 'LUMBO' (Lund University Mobile Biosphere Observatory) (Fig. 8.1). This unit will be used to develop LIDAR techniques for small flying insects such as *Culicoides*.

If we are able to record the position, sex and heading of small flying insects in the field in the near future, it will open up for a new era in entomological field studies. Of course it will be necessary to carry out exhaustive validation before such a system is ready, and it will also be necessary to deal with large amounts of digital data from each study. But once established, it will be possible to study the behavior of many species directly without interference.

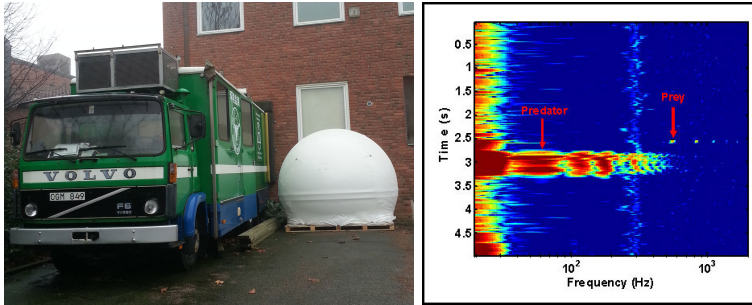


Figure 8.1: Left: The LIDAR truck with the mobile unit used to detect damselflies in the field and a newly arrived dome that will be a part of the 'LUMBO' unit. Right: A preliminary study carried out by Mikkel Brydegaard in Nairobi, 2012, where a mosquito (prey) is chased by a dragon fly (predator) and detected by remote scattering modulation spectroscopy at a distance of 120 m. Photo and graphics: Mikkel Brydegaard.

Thus, in the future, we will most likely be able to utilize more powerful and precise techniques for investigating small flying insects. However, the epidemiological purpose remains the same: To reveal patterns in space and time.



Figure 8.2: The author standing by the pump monument where John Snow had the handle removed and thus stopped the cholera outbreak in 1854. The famous pub named after John Snow is seen in the background. Photo: Nigel Bøttiger.





# Spatial abundance and clustering of *Culicoides*

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In press, *Parasites & Vectors*, 2013.

## Spatial abundance and clustering of *Culicoides* (Diptera: Ceratopogonidae) on a local scale

Carsten Kirkeby, René Bødker, Anders Stockmarr and Peter Lind

### Abstract

**Background:** Biting midges, *Culicoides*, of the Obsoletus group and the Pulicaris group have been involved in recent outbreaks of bluetongue virus and the former also involved in the Schmallerberg virus outbreak in northern Europe. For the first time, we here investigate their local abundance pattern on a field by intensive sampling with a grid of light traps on 16 catch nights. Neighboring trap catches can be spatially dependent on each other, hence we developed a conditional autoregressive (CAR) model framework to test a number of spatial and non-spatial covariates expected to affect *Culicoides* abundance.

**Results:** The distance to sheep penned in the corner of the study field significantly increased the abundance level up to 200 meters away from the sheep. Spatial clustering was found significant but could not be explained by any known factors, and cluster locations shifted between catch nights. No significant temporal autocorrelation was detected. CAR models for both species groups identified a significant positive impact of humidity and significant negative impacts of precipitation and wind turbulence. Temperature was also found significant with a peak at just below 16 degrees Celcius. Surprisingly, there was a significant positive impact of wind speed. The CAR model for the Pulicaris group also identified a significant attraction to smaller groups of sheep placed on the field. Furthermore, a large number of spatial covariates which were incorrectly found significant in ordinary regression models were not significant in the CAR models. The 95% C.I. on the prediction estimates ranged from 20.4% to 304.8%, underlining the difficulties of predicting the abundance of *Culicoides*. **Conclusions:** We found that significant spatial clusters of *Culicoides* moved around in a dynamic pattern varying between catch nights. This conforms with the modeling but was not explained by any of the tested covariates. The mean abundance within these clusters were up to 11 times higher for the Obsoletus group and 4 times higher for the Pulicaris group compared to the rest of the field.

**Keywords:** *Culicoides*, spatial clustering, local scale abundance, abundance modeling, spatial autocorrelation, bluetongue, Schmallerberg virus.

## A.1 Introduction

Since the incursion of bluetongue virus into northern Europe and the subsequent  
 960 discovery of Schmallenberg virus in the same region, *Culicoides* populations on  
 farms have become important for epidemiological research. Species of the Ob-  
 soletus group and the Pulicaris group are suspected to play an important role  
 in north European outbreaks of bluetongue and are found throughout northern  
 Europe [23, 38, 25, 27, 98]. Recently, it was confirmed that species in the Obsole-  
 965 tus group can replicate Schmallenberg virus [108]. Many large-scale studies and  
 transmission models have included spatial estimates of the abundance of *Culi-  
 coides* in Europe ([109, 106, 116, 1, 21, 34, 62, 107, 118, 117]), but few studies  
 have investigated the spatial pattern of *Culicoides* abundance on a local scale:  
 In 1951, Kettle [74] found that the abundance of *C. impunctatus* decreased pro-  
 970 portionally with distance to their breeding sites. This species is not dominant on  
 farms but frequently associated with bogs (e.g. [104]). Later, Kettle [75] found  
 indication of higher abundances of *C. impunctatus* and *C. pulicaris* L. near hosts  
 (cattle, horses and humans). Garcia-Saenz *et al.* [46] found a positive correla-  
 tion between the number of sheep near a light trap, and the number of female  
 975 *C. obsoletus* caught in the trap. Rigot *et al.* [112] found that the abundance of  
 different species of *Culicoides* were positively correlated with closeness to farms  
 in Belgium. In a large scale study, Purse *et al.* [104] found that the abundance  
 of adult *C. pulicaris* sensu stricto was correlated with vegetation indices, land  
 use and elevation above sea level; *C. punctatus* abundance was correlated with  
 980 the presence of sheep, temperature, land use and vegetation; and the abundance  
 of *C. obsoletus* was only correlated with temperature. Also, the abundance of  
 adult *C. impunctatus* was found to have a negative correlation with the pres-  
 ence of cattle, which might be because of their breeding sites (bogs) that are  
 often located away from cattle. Remote sensing can be used to estimate the  
 985 abundance of *Culicoides* (e.g. [106, 63, 104]), but provides only estimates of  
*Culicoides* abundance on a rough scale. In this study we take a novel approach,  
 using local-scale abundance data to investigate possible spatial and temporal  
 covariates for prediction of *Culicoides* abundance within a field.

Neighboring insect traps can be spatially dependent on each other (e.g. [102],  
 990 [30]), and [111] found significant overlapping catching areas between 8 W On-  
 derstepoort traps situated 50 meters apart. Thus it is necessary to take spatial  
 autocorrelation into account. We developed conditional autoregressive (CAR)  
 models for the abundance of two *Culicoides* vector species groups in order to  
 account for the spatial dependency. For the first time, spatial autocorrelation is  
 995 incorporated in a prediction model for *Culicoides* on a local scale, making trap  
 catches spatially independent by including information from neighboring traps.  
 Using this approach, a number of spatial covariates which have a significant  
 impact in ordinary regression modeling, no longer appear significant and some

temporal covariates become significant. At the same time we provide a method to deal with a lot of missing data in a spatial dataset by including second order neighbors when first order neighbors are missing. Furthermore, we estimate the spatial autocorrelation between trap catches and demonstrate the need to take it into account by incorporating it into statistical models. Lastly, we examine the abundance pattern not explained by the systematic part of the CAR models through cluster analysis.

## A.2 Field Data

The study site was an approximately 750 m long and 250 m wide field grazed by sheep in Denmark (Fig. 1, GPS coordinates: N55.3961, E12.1903), and the study period covered 7 weeks in June to August, 2009 (Table 1). The vegetation on the field was grasses and shrubs (about 10-30 cm height) and the field was completely surrounded by windbreaks consisting of trees and bushes (about 3-5 m height). No confounding light sources outside the field were visible at night. The surroundings were agricultural fields, except in the southern end and the north-western end of the field where there was tree cover. Fifty CDC Mini UV-light traps (John W. Hock, USA) were set up at a height of 180 cm in heavy metal gallows in 50 by 50 meter grid points covering the study field to measure the abundance of *Culicoides*. The grid size was chosen to sample the field evenly with little potential overlap of trap ranges [46]. For convenience we chose the CDC type traps and not the more commonly used Onderstepoort type trap. The CDC type traps are ideal for operation in the field using a 6 V battery and equipped with a photoswitch to save battery during the day when *Culicoides* are inactive. The traps turn on automatically at dusk and off at dawn. During the study period, 260 sheep (25-30 kg) had access to the whole field during the day, and were confined to a small enclosure in the northern end of the field before dusk until after dawn. This ensured that host animals were not present on the field at night and enabled a precise measure of the distance from each trap to the host animals.

Four potential breeding sites (A-D on fig. 1) for the *Pulicaris* group were subjectively identified on the field [96]: Site A was a shallow assembly of water without any boundary vegetation and a 1-3 meter broad mud zone; Site B was an old marl pit with shallow water, heavily shaded by dense thicket with trees; Site C was a small pond with reed along the steep edges; site D was a muddy area on the field with small temporary water bodies. Throughout the study period, twenty to fifty (according to area size) mud samples (97 mm in diameter) were taken weekly from each potential breeding site and kept in emergence chambers at an indoor facility (following [76]) to confirm breeding. Outside the potential

breeding sites, an additional 50 soil samples were taken weekly at randomly generated coordinates to screen for *Obsoletus* group breeding sites and for unexpected breeding sites of the *Pulicaris* group. We did not target breeding sites of the *Obsoletus* group as they are poorly investigated. They are associated with factors that are difficult to include in a model such as dung heaps and leaf litter, but this topic is still largely uncovered [136, 137, 99].

On three nights, sheep were placed in a transect in the middle of the field to test the attraction effect of a few sheep compared to the flock. In three transect points, two sheep were placed together in a 3 by 3 meter enclosure under a light trap (see fig. 1). The distance between transect points was 150 meters. During the study period, a weather station (Davis Vantage Pro 2) with a data logger was set up to record temperature, precipitation, humidity, wind speed and wind direction at 5 minute intervals. It was placed in the middle of the field to keep away from interfering vegetation. Light traps were emptied at dawn, and the caught *Culicoides* were preserved in 70% ethanol. The samples were analyzed under a dissection microscope, and sorted to species group and sex following Campbell and Pelham-Clinton [22]. Only females of the *Obsoletus* group (comprising *C. obsoletus*, *C. scoticus*, *C. chiopterus* and *C. dewulfi*) and the *Pulicaris* group (here comprising *C. pulicaris* and *C. punctatus*), were included in this analysis. We only considered the two dominant species in the latter group since other members of this group are rare in farm areas (pers. obs.) and not identified as a disease vector in this region. Due to time constraints, on 8 of the catch nights we only counted 50% of the trap catches. On these catch nights (the dates are underlined in Table 1), every second sample, chosen in a checkerboard pattern, was analyzed. All 16 nights were included in the models.

To deal with a high number of low catches we stabilized the observations by transforming the numbers with the natural logarithm prior to analysis,  $\log(x+1)$ . Thus for low numbers the observations will converge towards 1 instead of zero. For simplicity, we here denote the transformation as  $\log(X)$  in the equations.

### A.2.1 Temporal Covariates

Only weather records during the flight periods of *Culicoides* (assumed to be one hour before to three hours after sunset and two hours before to one hour after sunrise) were used in the analysis because we assume that the trap catches were only directly affected by the weather in this time interval. Mean temperature, humidity and wind speed measurements recorded on the field during the flight periods were included directly as covariates. Precipitation was summed over each flight period and included as a covariate. As an estimate of the wind turbulence, changes in wind direction was defined in steps as a minimum change

of wind direction of 22.5 degrees. The highest number of steps that the wind direction changed in either 5 or 10 minute intervals, measured within each flight period, was calculated. As each catch night consisted of two flight periods, the mean of the two highest step change numbers for each flight period was used as the turbulence covariate for each catch night.

### A.2.2 Spatial Covariates

The Euclidean distance from each trap to the sheep enclosure was used as a covariate. The inverse distance, squared inverse distance, log distance and the square of the log distance were also included in the initial models. We hypothesized that *Culicoides* could take advantage of shelter from the wind behind windbreaks surrounding the field. To construct this effect of windbreaks, the angle difference between the wind direction and the windbreak angle was found. The covariate was then equal to sinus to the angle, resulting in full effect of windbreaks perpendicular to the wind direction, and no effect of windbreaks parallel to the wind direction. Furthermore, the effect of a windbreak was only included if the wind blew towards the field through the windbreak. The windbreak effects were then multiplied by the inverse distance from each trap to the respective windbreaks. For each trap, the sum of all windbreak effects was used in the analysis. An effect of sheep scent was modeled in a similar way, using the sine function on the angle difference between the wind direction and the fence separating the sheep from the field. This corresponded to the odor-seeking function used in the model of Sedda *et al.* [118]. On the three catch nights where sheep transects were set up, the inverse Euclidean distance from each trap to the transect points was included as a covariate. The inverse squared distance from each trap to the nearest breeding site was tested to account for the effect of breeding sites. The following interactions between covariates were also tested: distance to sheep and windbreak effect, sheep scent effect and windbreak effect, wind speed and windbreak effect, wind speed and sheep scent effect. Squared relationships were included to allow for non-linear effects. A systematic effect of each catch night was also included in the model.

### A.2.3 Ordinary Regression Modeling

We first build a linear regression model for each of the two species groups, using backwards 1-step reduction from a model including all covariates:

$$\log(X) \sim \beta^T Z + \epsilon \quad (\text{A.1})$$

Where the log of the abundance of *Culicoides* ( $X$ ) is determined by covariates  $Z$  and their coefficients  $\beta$  (where  $\beta^T$  signifies the matrix transpose of  $\beta$ ), and a residual error term  $\epsilon$ . Model reduction was performed with the likelihood ratio method, and covariates that did not contribute significantly ( $p \geq 0.05$ ) were excluded. After model reduction, all excluded covariates were tested again by forward selection, with the test sequence defined through the Akaike Information Criterion (AIC) [2]. These models treated the trap catches as stochastically independent of each other and hence ignored potential spatial autocorrelation (clustering). All regression modeling was carried out in R 2.14.2 (www.r-project.org).

### A.2.4 CAR Modeling

To account for the spatial autocorrelation within the dataset, a conditional autoregressive model (CAR) model was constructed for each species group by assuming spatial dependence in the model (1) as described in the following. The model estimation and test procedure is described in Supporting Information, S1.

In order to transform these spatially dependent observations into a series where standard estimation techniques could be applied, the traps were first listed in a specific sequence, the *conditioning series*, starting with the trap in the upper right corner of the field and continuing straight down (see fig. 1), then moving left along the bottom of the field, one step up to the next trap and continuing straight up, then left along the top of the field and so on. For these sequential data, the following model was defined:

$$\log(X) \sim \beta^T Z + \varphi(\rho)N + \epsilon \quad (\text{A.2})$$

Where the log of the abundance of *Culicoides* ( $X$ ) is determined by the following components: The effect parameter matrix  $\beta$ , the vector of covariates  $Z$ , and the correlation matrix  $\varphi(\rho)$  capturing the effect of neighbors as a function of the spatial autocorrelation  $\rho$ , multiplied by the model's residual values  $N$  for the specific neighbor configuration for each trap catch (for neighbors with higher index in the conditioning series).  $\epsilon$  denotes the residual error term. Equation (2) was based on the theoretical spatial autocorrelation framework using block design by Besag [14], and by definition assumes that each observation is independent of all other observations given the first order neighbors, when these are all present. First order neighbors to a trap (with the trap in position 1 on fig. 2) comprised all trap catches at 50 meters distance to the trap on the same catch night (position 2 and 3 on fig. 2). Second order neighbors were defined, for use



in the estimation process when first order neighbors were missing, as first order neighbors to a first order neighbor, but not identical to the original trap (e.g. position 5, 6 and 7 are second order neighbors to position 1 through position 3 on fig. 2). The correlation between the traps and any first order neighbor was modeled as a constant  $\rho \geq 0$ . This, together with the requirement of conditional independence, defined the correlation structure between all traps, and thus  $\varphi(\rho)$  in equation 2, uniquely. For example, the correlation between a trap and a second order neighbor along a line transect was then  $\rho^2$ . Thus the model implies exponentially decreasing dependence between traps along line transects if first order neighbors are missing. This model is different from a normal CAR model in that the regression is weighted with different variances for each spatial neighbor configuration. The configuration of first and second order neighbors to each trap, and thus  $\varphi(\rho)$ , varies considerably in this analysis due to many missing observations. The standard error of  $\rho$  was estimated through the Fisher Information ([44]).

To evaluate the performance of the CAR models, the models were examined for significant spatial clusters in the residuals using a normal distribution model in SaTScan v. 91.1.1 ([www.satscan.org](http://www.satscan.org)). For each catch night and each species group, the model residuals were tested for circular or elliptic hotspots or coldspots, without penalty for elliptic clusters and allowing multiple hotspots. Each scan was run for 9999 iterations, testing for significant clusters at the 5% level. To investigate the spatial autocorrelation pattern not explained by the systematic part of the CAR models, we adjusted the observations for the significant effects found in the CAR models and then tested each catch night for significant clusters in SaTScan. Ordinary regression model and CAR model fit were tested by plotting the distribution of residuals and by quantile-quantile-plots.

### A.3 Results

During the study period, successful catches from 16 nights, consisting of 530 trap catches, were included in the analysis. A total of 19,654 female *Culicoides* were counted: 15,166 from the *Pulicaris* group and 4,488 from the *Obsoletus* group (table 1). The parameter ranges within the active period were: mean temperature: 12.1 - 19.9 degrees Celsius, mean wind speed: 0.08 - 5.47 m/s, precipitation: 0 - 3.6 mm, Relative humidity: 54-100%. The distance from each trap to the sheep was 38 - 653 meters and the distance to the nearest breeding sites 1-45 meters. Catches were excluded if the sheep broke through the enclosure during the night; the trap was damaged or not operating properly.

A total number of 208 *Culicoides* spp. hatched from the emergence chambers, of

which 16 were from the Pulicaris group and none were from the Obsoletus group. The other species that hatched were mostly *C. pictipennis* and *C. festivipennis*. From breeding site A, 24 *Culicoides* spp. (none were from the Pulicaris group) emerged from 350 soil samples. From the shaded breeding site B (fig. 1) no *Culicoides* but many Psychodidae spp. emerged from 140 soil samples. From breeding site C, 152 *Culicoides* spp. (of which 13 were from the Pulicaris group) emerged from 140 samples. From breeding site D, 32 *Culicoides* spp. (of which 3 were from the Pulicaris group) emerged from 140 samples. No *Culicoides* emerged from the 350 random samples on the field, indicating that Pulicaris group breeding sites were confined to the identified breeding sites and that the Obsoletus group did not emerge on the field during the study period. Distance to breeding sites A, C and D was included in the modeling procedure as they were found to be breeding sites for *Culicoides*.

The temporal autocorrelation between sampling nights was tested in the CAR models, and in both models it was found to be insignificant (Obsoletus group model:  $p=0.51$ , Pulicaris group model:  $p=0.76$ ). The spatial autocorrelation was highly significant ( $p$ -values: Obsoletus group model:  $p<0.0001$ , Pulicaris group model:  $p<0.0001$ ), and was estimated to be 0.41 ( $\pm 0.09$ ) for the Obsoletus group model and 0.235 ( $\pm 0.09$ ) for the Pulicaris group model for traps placed with 50 m distance. The residual variance in the Obsoletus CAR model was 0.69 and in the Pulicaris CAR model 0.65 (table 3).

The ordinary regression models without spatial autocorrelation identified more significant spatial covariates than the CAR models did, and the CAR models identified more temporal covariates than the ordinary regression models (table 3).

The CAR models for both species groups showed increased abundance of *Culicoides* near the sheep (t-test  $p$ -values for both models: distance to sheep  $< 0.001$ , squared distance to sheep  $< 0.001$ ). The mean abundance for the Obsoletus group was approximately twice as high near the sheep as 372 meters away where the minimum abundance level was found (fig. 3). The Pulicaris group abundance was approximately 1.5 times higher near the sheep than at 316 meters distance where the minimum abundance level was found. For both species groups, this effect was significant until 200 meters from the sheep, judged by visual inspection of the confidence limits on FIG.3. For both species there was an increase in the abundance estimate from 300 to 650 meters distance.

For both species groups we found a significant positive effect of humidity and a significant negative effect of turbulence and precipitation (Table 3). There was also a significant effect of temperature and wind speed including their squared terms. The temperature effect showed peak abundance at just below 16 degrees Celcius and the wind speed surprisingly showed a positive effect with increasing

wind speed between 1 and 5 m/s (Fig.3).

Only the CAR model for the Pulicaris group identified a significant effect of the transect of sheep on three catch nights. The effect of the inverse Euclidean distance to the small enclosures with pairs of sheep is positive, meaning that the Pulicaris group abundance is higher close to the pairs of sheep.

We tested for clusters in the residuals of the CAR models to check if the spatial autocorrelation was fully extracted in the models. In the residuals of the Obsoletus group CAR model we found two clusters ( $p=0.0045$  and  $0.0006$ ) on the nights of 28.08 and 31.08. We also found two clusters ( $p=0.0375$  and  $0.0427$ ) in the Pulicaris group CAR model on the nights of 06.08 and 31.08.

To investigate the spatial clustering pattern of vector abundance not explained by the systematic covariates in the CAR models, we subtracted the CAR model effects from the observations and tested for clusters using SaTScan. This procedure extracted the significant effects found in the CAR models without extracting the spatial clustering from the data, allowing us to examine the unexplained abundance pattern. Eight significant hotspots (mean trap catch ratios for catches within versus catches outside clusters: 2.70; 4.48; 2.57; NA; 10.82; 0.62; NA, where NA indicate an error caused by zero catches) and four significant coldspots (ratios: 0.32; 0.06; NA; NA) were found in the Obsoletus group data. In the Pulicaris group data, three hotspots (ratios: 1.75; 4.16; 1.95) and two coldspots (ratios: 0.52; 0.17) were identified (Figs. 4 and 5). In the Obsoletus group, four of the hotspots were found in the northern part of the field, three in the middle and one in the southern part. Also for this group there were three coldspots in the northern part and one in the southern part. One of the hotspots in the Pulicaris group data was found in the northern part, one in the middle and one in the southern part of the field. The two significant coldspots were located both in the northern and the southern part. Some of the traps were included in both hotspots and coldspots, which is a consequence of the SaTScan method forcing the cluster to be circular or elliptic. This highlights the short distance between hotspots and coldspots on the field. The significant hotspots and coldspots are placed similarly but not identically in the two species groups.

## A.4 Discussion

We tested the observations for clustering without the effect of host animals to investigate the spatial clustering pattern not explained by the systematic covariates in the CAR models. It revealed a dynamic pattern with higher *Culicoides* abundance in different places, varying between catch nights, so clusters were

moving around on the field (Figs. 4 and 5). This is consistent with the CAR modeling, and implies that one or more yet unidentified factors influenced the *Culicoides* abundance in a spatial pattern that changes each night. The ratios of the significant hotspots show that the mean abundance of the Obsoletus group in a significant hotspot was 0.62-10.82 times higher than the rest of the field (Fig. 4), and 1.75-4.16 times higher for the Pulicaris group (Fig. 5). This result is striking and can seriously impact field studies of *Culicoides* abundance. Since no known factor could explain this dynamic pattern, it will cause noise in abundance studies. The best way to take account for this is to conduct large-scale studies with many traps and locations reducing the noise from the spatial clustering. It is not possible to obtain a reliable measure of the level of abundance in an area by using a single trap. However, this does not mean that national or regional scale predictive abundance models are invalid if they are based on just one trap per farm. If a large number of farms are sampled, the general relationship between environmental factors and the mean abundance can still be quantified. Such models may therefore be able to predict a mean trap collection on farms associated with a specific combination of environmental covariates (e.g. [34, 106, 21]). But if the same models are used to predict catch sizes in a trap at a specific farm it may result in very large residuals as a result of the large spatial variation in abundance on the same farm.

In this study we found that the spatial autocorrelation between traps was highly significant. This means that if a trap catches more than expected, another trap close by is also likely to catch more than expected. For the Obsoletus group the spatial autocorrelation was 0.41. We explored this further (using equation (5), see Supporting Information, S1), to have a look at the relation between two traps, A and B, with 50 m distance. For an expected level of abundance at 100% in both traps, if trap A catches 20% more than expected, then trap B will be expected to catch 7.8% more. If trap A catches 50% more than expected, trap B will be expected to catch 18.1% more. For the Pulicaris group the spatial autocorrelation was 0.235. Using the same scenarios, trap B would catch 4.4% and 10.0% more than expected, respectively. The spatial autocorrelation means that traps placed close to each other do not provide independent estimates of abundance. The true variance in abundance will therefore be underestimated unless traps are widely separated. This has to be taken into account when using more than a single trap at a site.

The CAR models should extract the spatial clustering from the data and therefore leave no significant clusters in the residuals. However, we found two clusters ( $p=0.0045$  and  $0.0006$ ) in the residuals of the Obsoletus group CAR model. The first cluster is on the night of the 28.08.2009 where only one specimen from the Obsoletus group was caught on the entire field, and thus we ascribe this cluster as an artefact. The second cluster in the Obsoletus group CAR model residuals on the night of the 31.08.2009 is also highly significant. We performed the pa-

parameter estimation again without this catch night and found similar estimates of the effects (data not shown). Thus we conclude that this model violation does not influence the general validity of the model. Two clusters were found in the Pulicaris group CAR model residuals with p-values only just below the significance level ( $p=0.0375$  and  $0.0427$ ). Therefore we do not doubt the general applicability of this model either. Furthermore, the SaTScan analysis used to detect clusters is not able to deal with the varying variance included in the model residuals created by differing neighbor configurations, making this test very rigid.

From the two CAR models, the residual variance was estimated to be 0.69 and 0.65 (table 3). We can use this variance to estimate the general 95% confidence intervals of the abundance estimates. Thus the 95% interval for Obsoletus group CAR model ranged from 20.4% to 304.8% of the predicted catch size. For the Pulicaris group CAR model the interval ranged from 22.6% to 289.4%. This highlights the huge variation in the catches. Estimates of vector abundance based on single traps are expected to vary dramatically depending on the exact position chosen for the trap. This high uncertainty associated with abundance estimates based on single traps needs to be taken into account when modeling the abundance of *Culicoides* on a greater scale and in simulation models of vectorborne disease that rely on vector abundance estimates.

The estimates of the significant effects in the models are fairly similar between the two species groups (fig. 3, table 3). This supports the results of the models and indicates that the effects found may be general for species of *Culicoides*. Especially the significant temporal covariates, which may be general for *Culicoides* because they are not influenced by host preferences.

The dynamic pattern is also fairly similar between the two species groups. Surprisingly, three of the significant hotspots for the Obsoletus group and two for the Pulicaris group were found in the southern part of the field, away from the sheep. A possible explanation for this is swarming behavior. Downes observed in 1955 [39] that different species of *Culicoides* swarm above certain markers such as cow dung, a dark cloth or other conspicuous objects. Both the Obsoletus group and the Pulicaris group have been observed swarming, and it is likely that swarming can blur the general abundance pattern. Very few males were caught in the light traps in this study, and they seemed to be correlated with high female abundance (data not shown), which could also indicate swarming behaviour.

Similar to the results from other studies [12, 46, 112], we found a significant effect of the vicinity of host animals for both the Obsoletus group and the Pulicaris group. In a study of Calvete *et al.* [21], traps were placed within 30 m from each farm to obtain estimates of the abundance of *Culicoides*, and Goffredo

and Meiswinkel [54] pointed out that when monitoring *Culicoides*, light traps should be placed in the near vicinity of vertebrate hosts. This is supported by the present study where we quantified the effect of host animals. We found that traps placed near host animals increased the overall vector abundance with approximately 50% - 100% compared with 300-400 m away from the host animals. However, we also found an increased level of abundance for both species groups in the southern part of the field. This could be an artefact in the simple two-parameter model construction, or it could indicate a depletion of *Culicoides* abundance around the host animals. In the latter case, the abundance level is normal again at 650 m distance from the host animals. An alternative explanation could be that this effect is caused by the small forest area in the southern part of the field.

This pattern is relevant for other studies of the abundance of *Culicoides*. Traditionally, *Culicoides* monitoring programmes are carried out running a single trap on each farm near host animals. Calvete *et al.* [21] mentions that traps were placed within 30 m from the hosts to ensure a high catch. Goffredo and Meiswinkel [54] suggest that traps are placed in the vicinity of hosts for monitoring programmes. We suggest, that the trap placement should be standardized or adjusted with regards to the distance to host animals because the distance to the hosts impacts directly on the trap catch. For instance, if placement of the traps just next to the host animals is impossible, all traps in a study should be placed at the same distance to obtain comparable measures at different farms. Alternatively, if one trap is placed sub-optimally at for instance 300 meters distance from the host animals, catches of the Pulicaris group made here should be adjusted up by 150%.

We also found a significant effect of the sheep placed in transects on the field for the Pulicaris group. This emphasizes that this species group are more abundant where the host animals are, and that even two sheep can have an impact on the abundance of this species group as found by Garcia-Saenz *et al.* [46]. It also underlines the fact that *Culicoides* can find any small group of host animals regardless of other groups of hosts nearby, which makes them very efficient disease vectors.

The temperature was significant for both species groups with peak abundance at 16 degrees Celcius and no effect below 14 degrees or above 18 degrees (fig. 3). This is in concordance with Conte *et al.* [34] who found that the minimum temperature for activity of the Obsoletus Complex was 14.2 (13.9 - 14.6) degrees Celcius. Garcia-Saenz *et al.* [46] found no significant effect of temperature on the abundance of *Culicoides*, but Carpenter *et al.* [26] found a peak biting rate at 21 degrees. The latter study included catches at temperature up to 29 degrees, which was not possible to include in the present study.

The humidity was found to have a positive significant effect on the abundance of both species groups. This is in concordance with Carpenter *et al.* [26] who found a positive correlation between humidity and *Culicoides* abundance. Carpenter *et al.* [26] and Baylis *et al.* [10] also found a positive effect of humidity on the abundance of the Obsoletus group. Turbulence had a significant negative effect in the CAR models for both species groups. Carpenter *et al.* [26] also found this significant effect. In the present study we also found that precipitation had a negative effect on the *Culicoides* abundance. This contrasts with the findings of Blackwell [18] who found a positive effect of rain on catches of *C. impunctatus*.

We found a significant effect of wind speed and its quadratic term for both species groups. When plotting with confidence intervals, the abundance increases with the wind speed in the investigated interval (Fig. 3). In contrast, Carpenter *et al.* [26] found decreasing abundance for wind speeds exceeding 3 m/s. A possible explanation of the findings in our study is that if the wind is weak and the *Culicoides* therefore have difficulties in determining the direction of hosts by scent, they are reluctant to waste energy on flying. Thus, within the investigated range of windspeed, higher wind speeds yield a higher abundance of active *Culicoides*.

No *Culicoides* emerged from breeding site B (Fig. 1). This could be due to the thicket and trees shading the pond, which prevents the sun from heating up the mud to the necessary temperature for *Culicoides* to breed. The other three sunlit breeding sites were expected as breeding sites for *Culicoides* spp. In this study we used light traps to measure *Culicoides* abundance. Therefore the results may be influenced by bias of the trapping method such as variation in attraction for different species and for different lifestages of *Culicoides* [26, 52, 132, 129]. Future trapping studies should ideally distinguish specimens to species level in order to determine the differences in the behaviour between species with regards to light traps.

The spatial autocorrelation,  $\rho$ , was found significant, meaning that it is necessary to take spatial clustering into account on this scale. Even on a larger scale, spatial clustering is important to incorporate in the modeling framework as shown by [117]. The temporal autocorrelation,  $\theta$ , was found non-significant. This was expected since the intervals between catch nights ranged from 0 to 10 nights. The ordinary regression models identified more significant spatial covariates than the CAR models, effects which the CAR models discarded through the inclusion of local dependence given by the spatial correlation (table 3). A possible explanation for the extra significant spatial covariates included in the ordinary regression models is that they compensate for the spatial clustering by including more explanatory covariates, and it should be noted that given the validity of the CAR model, these significances are type 1 errors, ie. false significances. This interpretation is further supported by the fact that the significant

covariates shared by the CAR models and the ordinary regression models are fairly alike (table 3). In the present study, the systematic effect of each catch night may have overtaken the effect of some of the covariates when few catch nights are sampled because non-spatial covariates will covary with catch night, which is a drawback of this type of model. However, the advantage is that we obtain more precise estimates of significant covariates corrected for the effect of spatial autocorrelation.

We used Besag's block design to build the CAR models in this study [14]. Formulating the spatial autocorrelation as an exponentially decreasing correlation between neighboring traps we were able to include data points where all first order neighbors were missing by taking second order neighbors into account. This approach is useful in studies of grid measurements where many missing data are present.

The spatial autocorrelation between trap catches,  $\rho$ , accounts for other potential unknown covariates which were not spatially consistent between catch nights. However, if an unknown, spatially fixed factor influenced the abundance of *Culicoides*, the temporal autocorrelation,  $\theta$ , would tend to be significant, indicating that some traps consistently caught higher numbers of *Culicoides*. But since the temporal autocorrelation was found insignificant and the spatial autocorrelation was found significant, there is no evidence for the presence of unknown spatially fixed covariates.

## A.5 Conclusions

We revealed a spatially varying pattern of abundance that varies between catch nights, where unpredictable hotspots caused the mean trap catch to be up to 11 times higher for the *Obsoletus* group and 4 times higher for the *Pulicaris* group. From the residual variance of the models we calculated that the 95% C.I. on the prediction of abundance is approximately 20% to 300%, which is important to consider when conducting large-scale studies. We found no significant spatial covariates determining the abundance of the studied species groups other than the distance to host animals and for the *Pulicaris* group this also included pairs of sheep placed in small enclosures on the field. Thus no low risk areas for placing host animals susceptible to bluetongue or Schmallenberg virus were identified on this scale because the abundance of *Culicoides* was indeed determined by the presence of host animals. We have demonstrated the importance of placing traps near the hosts when monitoring *Culicoides*, as we see a significantly increased abundance of *Culicoides* (up to 100%) in a radius of approximately 200 meters from the hosts. We also found significant positive effects of humidity and wind



1455 speed, significant negative effects of precipitation and turbulence. The optimum  
temperature for abundance of both species groups was found to be just below  
16 degrees Celcius.

## A.6 Competing interests

The authors declare that they have no competing interests.

## 1460 A.7 Author's contributions

This project is a main part of the PhD project by Carsten Kirkeby at the  
Veterinary Institute at the Technical University of Denmark. Carsten Kirkeby  
conceived the study, carried out the planning, the field work, the analysis, con-  
tributed to the parameter estimation technique and wrote the manuscript. René  
1465 Bødker participated in the planning, analysis and discussion of the results. An-  
ders Stockmarr participated in the planning of the field work, provided the  
technique for the parameter estimation and took part in the analysis and the  
discussion of the results. Peter Lind participated in the discussion of the results.  
All authors read and approved the final version of the manuscript.

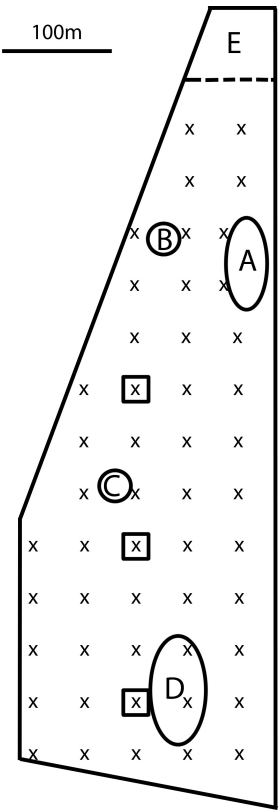
## 1470 A.8 Acknowledgements

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the EDENext Steering Committee as EDENext 065 (<http://www.edenext.eu>).  
The contents of this publication are the sole responsibility of the authors and  
do not necessarily reflect the views of the European Commission.

A.9 Figures

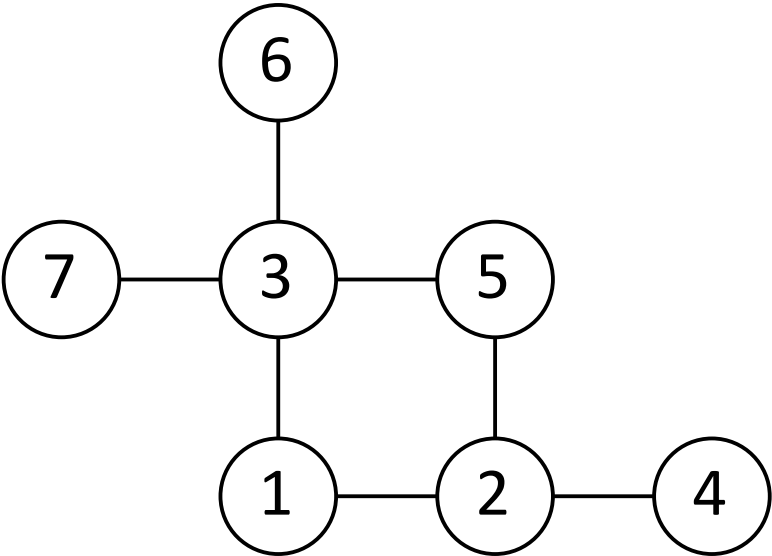
A.9.1 Figure 1 - Study site

1480



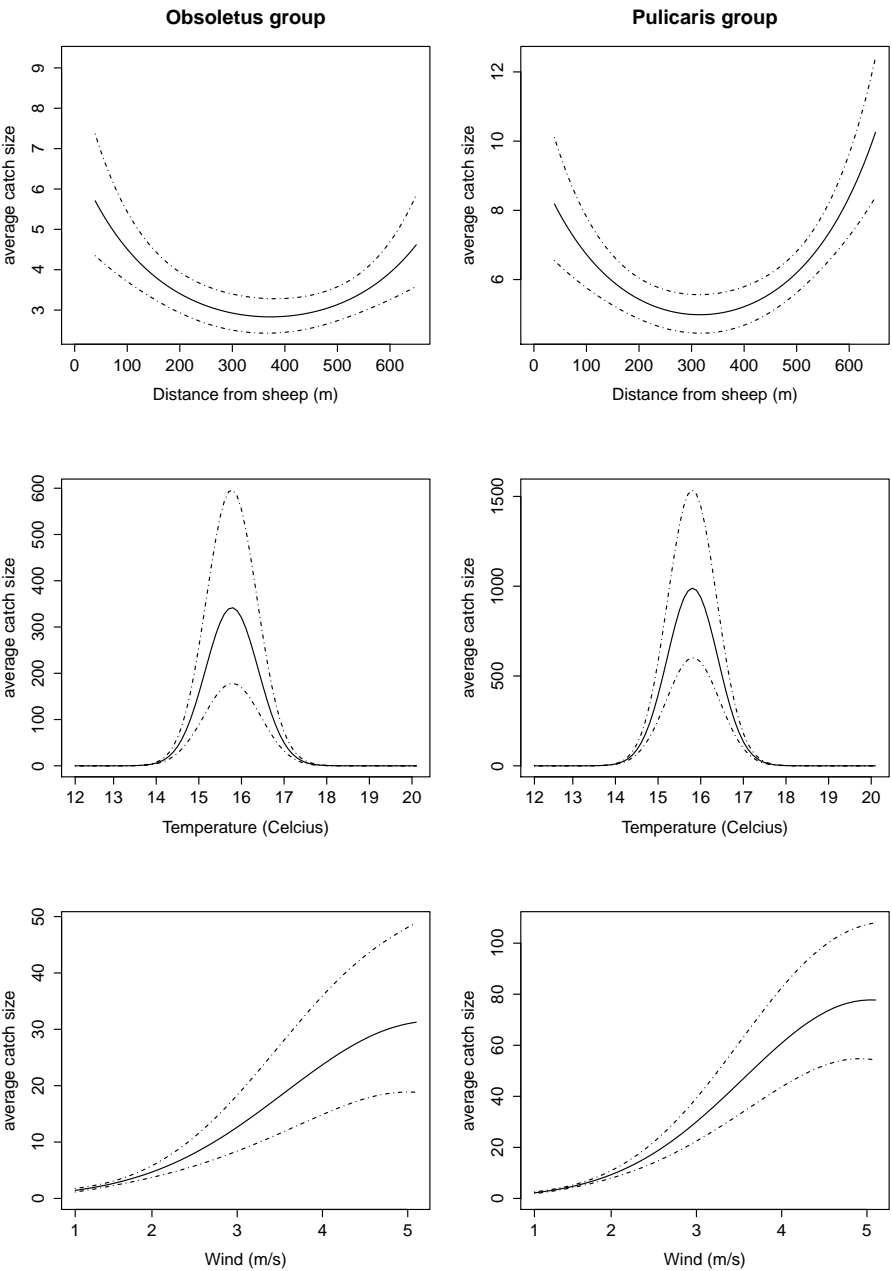
Outline of the study field with potential breeding sites (A, B, C, D) and the enclosure where the sheep were kept at night (E). Trap positions are marked with X and square boxes represent small enclosures for the transect experiment.

A.9.2 Figure 2 - Neighbors



A scheme of the relationship between a trap (position 1) and its first order neighbors (positions 2 and 3) and second order neighbors (positions 4, 5, 6 and 7). This diagram covers all possible situations because the autocorrelation is estimated by successively conditioning, meaning that only neighbors for which the trap catch have not been conditioned before will be counted as neighbors.

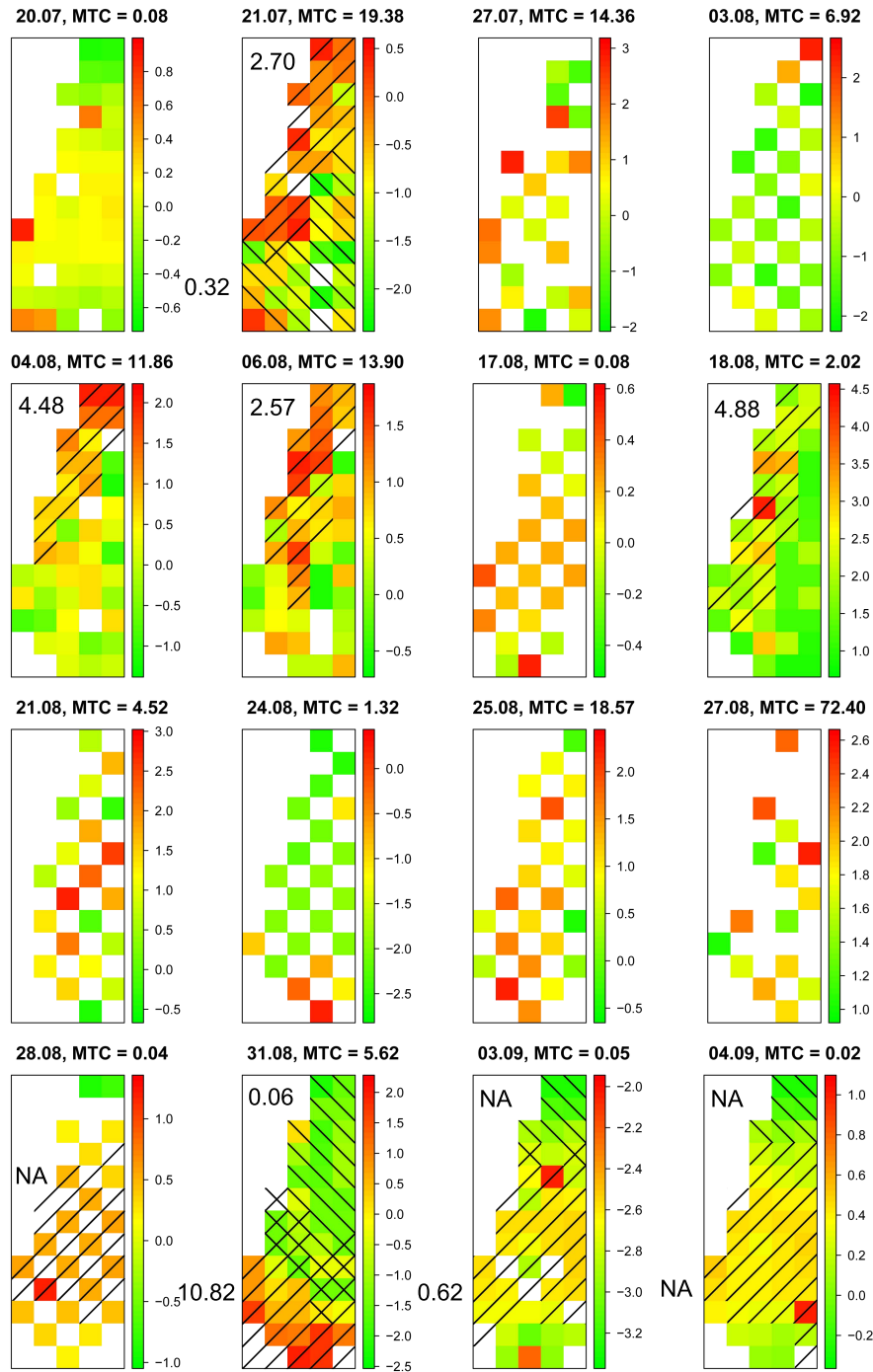
A.9.3 Figure 3 - CAR model effects



General effect of the distance to the sheep, temperature and wind, resulting from the CAR models. Plots show the mean effects in the investigated intervals with 95% confidence intervals. The functions are shown in the investigated interval and the curves will be vertically shifted between catch nights. For both species groups, the level of abundance is above the 95% confidence limits for the distance with minimum catch up to 200 meters from the sheep. Also for both groups, there is a peak activity at just below 16 degrees.



A.9.4 Figure 4 - Obsoletus group abundance pattern

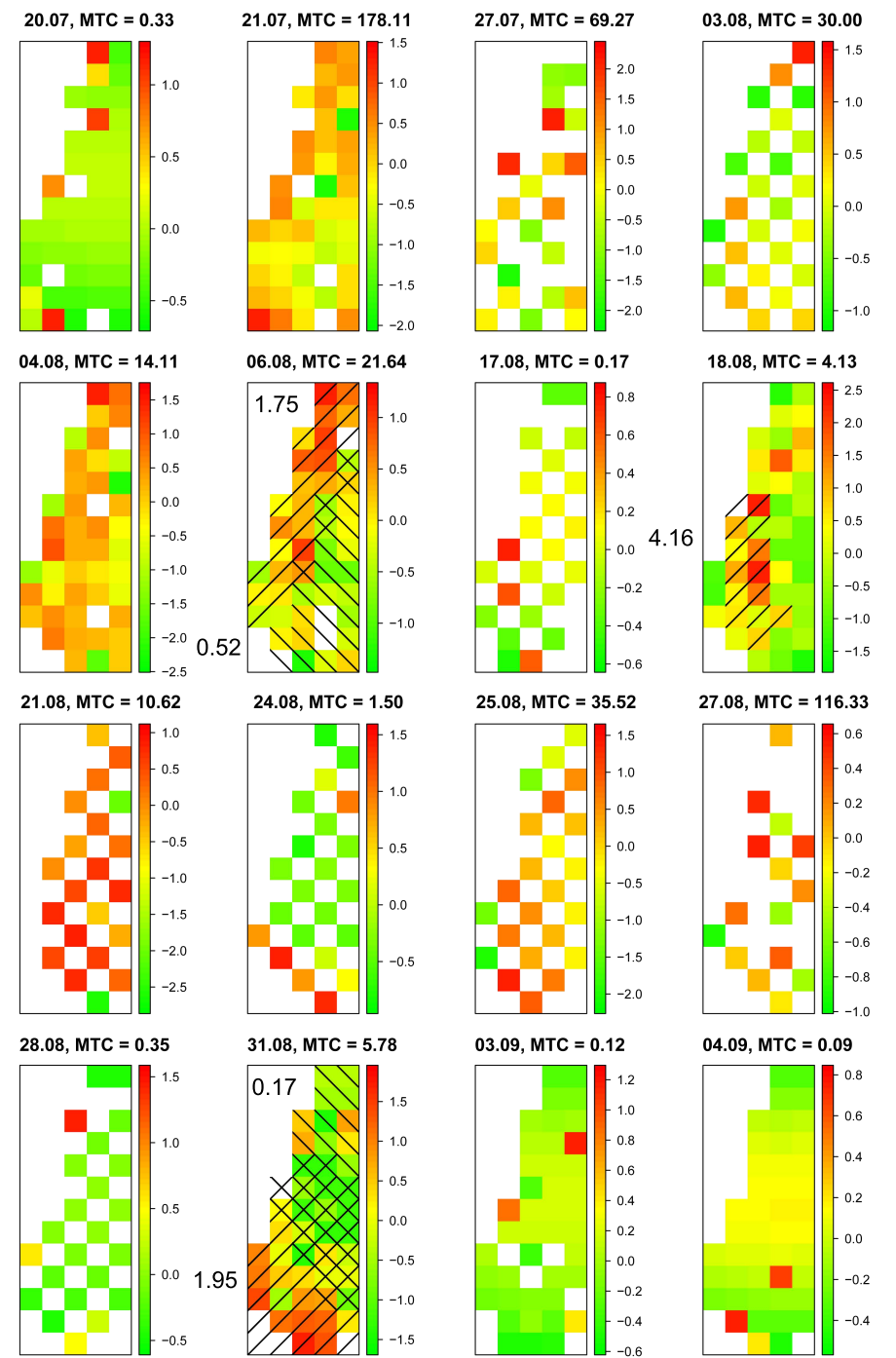


Visualization of the spatial clustering left in the data when the CAR model effect has been subtracted. Maps show the log of trap catch size for the *Obsoletus* group each catch night without the effect of distance to host animals. Traps that are included in significant hotspots are right-hatched and those in significant coldspots are left-hatched. The mean abundance ratio is noted for each cluster. MTC = mean trap catch per catch night. Note that the hotspots are moving around from catch night to catch night, and that some of the hotspots are similar to Fig. 5. The low hotspot ratio on the night of the 3rd September is an artifact caused by low catch numbers.





A.9.5 Figure 5 - Pulicaris group abundance pattern



Visualization of the spatial clustering left in the data when the CAR model effect has been subtracted. Maps show the log of trap catch size for the *Pulicaris* group each catch night without the effect of distance to host animals. Significant traps that are included in significant hotspots are right-hatched and those in significant coldspots are left-hatched. The mean abundance ratio is noted for each cluster. MTC = mean trap catch per catch night. Note that the hotspots are moving around from catch night to catch night, and that some of the hotspots are similar to Fig. 4.

A.10 Tables

Table 1:  
The total number of *Culicoides* caught on each successful catch night and the temporal covariates. On the underlined dates, half of the samples were not analyzed.

Date of sampling night	20.07	21.07	27.07	03.08	04.08	06.08	17.08	18.08
Obsoletus group total	4	872	316	173	522	612	2	93
Obsoletus group min/max	0/1	2/79	0/68	0/106	1/79	2/48	0/1	0/20
Pulicaris group total	15	8015	1524	750	621	952	4	190
Pulicaris group min/max	0/5	18/914	5/323	7/128	0/65	6/80	0/2	0/27
Wind speed (m/s)	1.4	2.7	1.4	2.4	0.5	0.2	2.9	2.5
Turbulence	0	0	0	0.2	0	0.2	0	0
Temperature (Celcius)	14.8	13.6	14.9	18.1	15.4	15.3	16.6	13.7
Mean humidity (%RH)	84.4	88.3	77.5	85.0	94.6	84.5	90.5	77.3
Precipitation (mm)	0.2	0.2	0	0.6	3.6	0	0	0.2

Date of sampling night	21.08	24.08	25.08	27.08	28.08	31.08	03.09	04.09
Obsoletus group total	95	29	427	1086	1	253	2	1
Obsoletus group min/max	0/18	0/12	2/58	24/176	0/1	0/44	0/1	0/1
Pulicaris group total	223	33	817	1745	8	260	5	4
Pulicaris group min/max	0/23	0/9	3/139	44/166	0/4	0/37	0/2	0/2
Wind speed (m/s)	3.3	1.8	3.7	0.9	0.1	1.3	0.9	5.5
Turbulence	0.5	0	0	0	0	0	0.1	0
Temperature (Celcius)	19.9	15.9	17.3	15.6	18.4	12.1	14.8	14.3
Mean humidity (%RH)	90.6	78.7	81.9	77.5	82.0	80.2	87.6	87.0
Precipitation (mm)	0	0	0	0	0	0	0	0.2

1530 Table 2:  
Mean, standard error and ranges of spatial covariates in the models.

	Mean	Variance	Range
Distance to Sheep (m)	390	30997	38 - 653
Breeding site effect	$4.7 \cdot 10^{-4}$	$9.6 \cdot 10^{-7}$	$3.6 \cdot 10^{-5}$ - $5.1 \cdot 10^{-3}$
Windbreak effect	0.048	0.019	0.001 - 1.383
Sheep scent effect	$4.5 \cdot 10^{-4}$	$6.97 \cdot 10^{-6}$	0.000 - 0.041

Table 3 (next page):  
Significant coefficients from the models. Insignificant covariates are denoted with '-'. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ , NS, not significant; NA, no estimate. Catch nights were tested together, and the significance is shown at each first catch night. The p-values for main effects includes the removal of both main effects and any interactions with this. No significances are given for the effect of catch nights, which were included as a systematic effect in all models.

	Ordinary regression			CAR models		
	Obsoletus group	Pulicaris group	Obsoletus group	Obsoletus group	Pulicaris group	Pulicaris group
Intercept	0.73	0.43	-349	-386		
Distance to sheep	$-4.5 \cdot 10^{-3}$	***	$-4.7 \cdot 10^{-3}$	***	$-4.05 \cdot 10^{-3}$	***
Distance to sheep <sup>2</sup>	$6.0 \cdot 10^{-6}$	***	$6.3 \cdot 10^{-6}$	***	$6.42 \cdot 10^{-6}$	***
Precipitation	NS	NS	-66.2	***	-73.40	***
Turbulence	NS	NS	-186.2	***	-206.6	***
Humidity	NS	NS	1.06	***	1.19	***
Temperature	NS	NS	39.95	***	43.91	***
Temperature <sup>2</sup>	NS	NS	-1.27	***	-1.40	***
Wind speed	$-7.6 \cdot 10^{-4}$	*	1.84	***	2.27	***
Wind speed <sup>2</sup>	NS	NS	-0.18	**	$-0.23 \cdot 10^{-2}$	***
Sheep transect	NS	0.51	NS	0.4794	*	
Windbreaks	-0.28	*	$-9.7 \cdot 10^{-2}$	***	NS	NS
Sheep scent	-7.116	***	0.81	*	NS	NS
Windbreaks * Sheep scent	$4.8 \cdot 10^3$	***	2077	*	NS	NS
Wind speed * Sheep scent	-84.96	*	NS	NS	NS	NS
Catch night 21.07	2.68		4.84		-0.56	1.03
Catch night 27.07	2.06		3.60		-4.24	-3.32
Catch night 03.08	1.09		3.05		59.64	67.31
Catch night 04.08	1.96		2.22		221.60	245.30
Catch night 06.08	2.37		2.81		21.86	24.59
Catch night 17.08	0.01		-0.04		-9.51	-10.76
Catch night 18.08	0.73		1.09		-5.48	-5.92
Catch night 21.08	1.34		2.11		108.80	120.60
Catch night 24.08	0.36		0.38		NA	NA
Catch night 25.08	2.72		3.17		NA	NA
Catch night 27.08	4.11		4.56		NA	NA
Catch night 28.08	-0.07		0.02		NA	NA
Catch night 31.08	1.09		1.26		NA	NA
Catch night 03.09	-0.05		-0.08		NA	NA
Catch night 04.09	NA		-0.06		NA	NA
Residual variance	0.68		0.65		0.69	0.65

## A.11 S1 (Supporting Information 1)

### A.11.1 CAR model estimation and testing

In order to estimate the parameters in the CAR model, we first set up a linear regression model corresponding to equation (1) including all covariates, with spatial dependence modeled through first and second order neighbors as described above. A product likelihood was found through successive conditioning of the full likelihood in the conditioning series, so that each trap catch was conditioned with all trap catches with higher index in the conditioning series. In the situation with missing first order neighbors, conditioning was performed on both first and relevant second order neighbors. The conditioning series impacted through first and second order neighbors with higher index, following the pattern in fig. 2 so that, using Besag's (1974) block design, only 1st order neighbors impact if none of these are missing, and 2nd order neighbors enters if any 1st order neighbors are missing. For example, if the 1st order neighbor in position 3 in Fig. 2 is missing, the 2nd order neighbors in positions 5, 6 and 7 will enter the list of neighbors on which conditioning is performed. If the 1st order neighbors at position 2 and 3 are both present,  $\varphi(\rho)$  is a  $1 \times 2$  matrix with values equal to  $2\rho/(1 + \sqrt{1 + 8\rho^2})$  at both entries. This transforms the model (1), with spatial autocorrelation, into the linear regression model for independent variables (2) by essentially including the spatial autocorrelation as a regression parameter. Parameter estimation was performed in a two-step procedure, where estimates of  $\beta$  were calculated conditionally on  $\rho$ , subsequently maximizing the profile likelihood for  $\rho$ . First we fixed a value  $\rho_0 \geq 0$  for  $\rho$ . Then the effects of neighbor observations  $\varphi(\rho_0)N$  in equation (2) was written as  $\varphi(\rho_0)(\log(X_N) - Z_N^T \beta)$ , where  $X_N$  is a vector containing the neighbor abundances, and  $Z_N$  is a matrix with each column containing the covariates for the corresponding neighbor. Now observe that:

$$\varphi(\rho)N = \varphi(\rho)(\log(X_N) - Z_N^T \beta) = \varphi(\rho)\log(X_N) - \beta^T Z_N \varphi(\rho)^T \quad (\text{A.3})$$

To write the model (2) on a standard form with independent variables, (2) is rewritten by splitting the term  $\varphi(\rho)N$  as described in (3), subtracting  $\varphi(\rho_0)\log(X_N)$  from (the log of) each observation and similarly subtracting  $Z_N\varphi(\rho_0)^T$  from each set of covariates, thus transforming the model (2) back to a model of the same form as (1), but differing from an ordinary regression model in that variances for the observations are not identical, but differ as the neighbor configurations differ. Estimation of  $\beta$  for  $\rho = \rho_0$  was then carried out by weighted linear regression with the variances acting as weights, thus taking within-neighbor correlation into account. In the event where successive conditioning yielded

only approximate independence, because neither 1st nor 2nd order neighbors were observed in full in the configuration in fig. 2, full independence was assumed for the estimation purpose. Taking the value of the likelihood function maximized for  $\beta$  this way as the value of the profile likelihood for rho at  $\rho_0$ ,  
 1585 the estimation procedure was completed by maximizing the profile likelihood to obtain simultaneous estimates for  $\beta$  and rho that yielded the maximum value of the likelihood function.

To test if there was significant temporal autocorrelation between the catch nights in the dataset, we expanded the developed model to include the trap catch from  
 1590 the previous catch night as a regression parameter,  $W$  in (3), with the coefficient  $\theta$ :

$$\log(X) \sim \beta^T Z + \theta W + \varphi(\rho)N + \epsilon \quad (\text{A.4})$$

Estimation was then performed as described above. The CAR models were reduced sequentially with the likelihood ratio test at a 5% significance level, and a forward selection procedure similar to the ordinary regression analysis  
 1595 was subsequently performed. To finally test if the spatial autocorrelation was significant, we set  $\rho$  to zero and tested if this model performed significantly worse than the developed CAR model (significance level = 5%). The same procedure was used to test if the temporal autocorrelation was significant, setting  $\theta$  to zero.

### 1600 A.11.2 Impact of spatial autocorrelation

To investigate the impact of spatial autocorrelation on two traps, A and B, placed with 50 m distance, we use this formula to find the adjusted expected level of abundance in trap B:

$$A_{B|A} = E_B * (O_A/E_A)^\rho \quad (\text{A.5})$$

Where  $A_B$  is the adjusted expected catch size of trap B given trap A,  $E_B$  is the general expected catch level of trap B,  $E_A$  is the expected catch level of trap A and  $O_A$  is the observed catch in trap A.  $\rho$  is the spatial autocorrelation at 50 m distance. Equation 5 is a consequence of the applied CAR model.





# **Spatio-temporal optimization of sampling for bluetongue vectors (*Culicoides*)**

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## Spatio-temporal optimization of sampling for bluetongue vectors (*Culicoides*)

Carsten Kirkeby, René Bødker, Anders Stockmarr and Peter Lind

### Abstract

Estimating the abundance of *Culicoides* using light traps is influenced by a large variation in abundance in time and place. This study investigates the optimal trapping strategy to estimate the abundance or presence/absence of *Culicoides* on a field. 45 light traps were used to sample specimens from the *Culicoides* obsoletus species complex on a 14 hectare field during 16 nights in 2009. The large number of traps and catch nights enabled us to simulate a series of samples consisting of different numbers of traps (1-15) on each night. We also varied the number of catch nights when simulating the sampling, and sampled with increasing minimum distances between traps. We used resampling to generate a distribution of different mean and median abundance in each sample. Finally, we used the hypergeometric distribution to estimate the probability of falsely detecting absence of vectors on the field. The variation in the estimated abundance decreased steeply when using up to six traps, and less pronounced when using more traps, although no clear cutoff was found. We found no general effect of increasing the distance between traps. We found that 18 traps were required to reach 90% probability of a true positive catch when sampling just one night. If two catch nights were available, only three traps per night were sufficient to reach the same probability level. The results are useful for the design of vector monitoring programmes.

**Keywords:** *Culicoides* obsoletus, spatial variation, light traps, abundance, bluetongue.

## B.1 Introduction

1635 Estimates of the abundance and presence/absence of vectors are essential for un-  
derstanding and modeling vector-borne diseases. Light traps is the most widely  
used method to sample *Culicoides* and single light traps are often assumed to  
be representative for abundance in a large area (e.g. [89, 104]). In a previous  
1640 study, substantial spatial clustering of *Culicoides* abundance was found within  
a field [77], where the abundance could be up to 11 times higher in the hotspots.  
In this study we analyse the data from that study from a practical perspective,  
quantifying the impact of variation in spatial abundance, relevant for studies  
using light traps.

## B.2 Materials and Methods

1645 In a field study we used 45 battery-operated CDC 4 W light traps ([www.johnwhock.com](http://www.johnwhock.com))  
on a field (length: 750 m, width: 250 m) near Vallø, Denmark, during the sum-  
mer (July - September) of 2009. A more detailed description of the study is  
given in Kirkeby et al. [77]. The traps were evenly dispersed throughout  
the field in 50 by 50 m grid points[77]. 260 sheep were confined to an enclosure  
1650 in one end of the field at night but had access to the full area during the day.  
Light traps turned automatically on at dusk and off at dawn. *Culicoides* were  
preserved in 70% ethanol each day. Only females of the Obsoletus group (com-  
prising *C. obsoletus*, *C. scoticus*, *C. chiopterus* and *C. dewulfi*) were included  
in the dataset. After the field study, on eight of the catch nights, only half  
1655 of the traps were sorted and counted due to time constraints. All statistical  
calculations were carried out in R (R 2.12.2).

A first analysis was carried out to quantify the sampling variation on a sin-  
gle catch night, and was repeated for all 16 nights. For each catch night we  
generated 10,000 random samples using one to 15 traps per sample. For each  
1660 combination of the 16 nights and 15 different numbers of traps we calculated  
the mean number of vectors of the 10,000 random samples, resulting in a dis-  
tribution of mean vector abundances for each sample size.

Given the spatial clustering on the field [77], a second analysis was performed to  
investigate if a minimum distance between the traps would improve the mean  
1665 abundance estimate. We resampled one to ten traps 2,000 times using three  
different minimum distances between the traps: 50 m; 100 m; and 150 m. We  
used the variation in mean abundance estimates to evaluate if more distance  
resulted in less variance. This was carried out on data from the nights of July

21<sup>st</sup> and August 31<sup>st</sup> where strong clusters were found on the field.

In a third analysis we quantified the probability of getting a false negative result (falsely detecting absence) using a given number of traps (1-20) per sample on the field. The mean of these probabilities, weighted with the analyzed number of traps per night, was used as the general probability for a negative sample during the whole study period. The hypergeometric distribution was then used to calculate the probability of detecting false absence for n (1-20) traps. We also removed five catch nights with more than 90% negative samples and repeated the calculations with this modified dataset.

Finally, a fourth analysis was carried out to identify the number of traps necessary to detect presence of vectors when using from one to ten traps on one, two or three randomly selected nights. Using the hypergeometric distribution as above, we determined the number of traps required for detecting the presence of vectors with 90% or 95% certainty. The number of traps required to reach the certainty levels when sampling on two and three nights were calculated by exponentiating the probabilities for one catch night to the power of two and three. This procedure was also repeated with the modified dataset. For each catch night we also calculated the probability of falsely detecting absence of vectors using one to ten traps, by exponentiating the probabilities as above.

### B.3 Results

A total of 16 catch nights were obtained during a 46 day period in the summer of 2009, from which 4,488 females of the *C. obsoletus* group were counted (Table 1). Out of total 530 samples, 224 (42%) were negative for *C. obsoletus* and 306 (58%) were positive.

In Fig. 1 (right), the range of the 95% simulation envelope decreased from using one trap (range: 2 to 48) with 50% when using six traps (range: 10 to 30). As expected, the vector abundance showed a declining sampling variation around the mean with an increasing sample size, most clear on nights with a high total catch (Fig. 1). When using only one trap, the median abundance was lower than the mean catch, demonstrating overdispersion of the catches.

The second analysis examined if a more precise abundance estimate may be obtained with larger distance between the traps in each sample. There was only a marginal decrease in the variance of the abundance estimate when sampling with one to ten traps using minimum 50; 100 and 150 m distance between traps (data not shown).

The results of the third analysis showed a strong decrease in the average probability of falsely detecting absence when increasing the number of traps (Table 2). Using the modified dataset remarkably lower probabilities were found. On the five catch nights with more than 90% negative trap catches (Table 1), there was only little effect of including more traps (Fig. 2 left). On the 11 catch nights with less than 90% negative trap catches three traps were mostly necessary to reach less than 5% probability of falsely detecting absence of vectors on the field (Fig. 2 right).

In the fourth analysis we calculated the number of traps required to reach less than 10% and 5% probability of finding a false negative result. Using more than one catch night remarkably reduced the necessary number of traps to reach these probabilities (Table 3).

## B.4 Discussion

In the present study we found that increasing the sample size from one to about six traps dramatically reduced the variation in the mean abundance estimate and reduced the difference between the mean and the median estimate. We found no obvious effect on the mean abundance estimate of increasing the distance between the traps. Thus the data suggest that, when sampling with more than one trap on a field, a better estimate of the abundance will not be obtained by placing traps with a large distance to each other.

The mean number of vectors caught in this study was 10.7 specimens per trap. Although the numbers of vectors caught in the present study were not as high as in other studies [7, 54, 89], the results can be used as a rule of thumb in other studies in Denmark and adjacent countries in northern Europe where the biological and meteorological conditions are comparable.

The present study represents a worst case scenario for presence/absence studies, i.e. where vectors are present but in low numbers and therefore difficult to detect, for instance when monitoring for a vector free period. On some catch nights the general abundance was very low, yielding a high probability of falsely detecting absence (Fig. 2 left). Thus we suggest sampling more than one night to minimize the impact of temporal variation. We also suggest avoiding catch nights with low vector activity by using e.g. weather forecasts [114], which will increase the certainty of detecting vectors if they are present in an area.

There are four main concerns about the spatial abundance in the data. Firstly, there was spatial autocorrelation (clusters) in the data, and the aim of the

present analysis was to address the practical implications of this effect.

1740 Secondly, we are resampling from a limited number of traps. This will cause the variation in samples with many traps to decrease more than if we were not restricted to a fixed number of traps.

1745 Thirdly, the range of attraction for the light traps is important: As shown in Kirkeby et al. [78], the maximal range of attraction for the CDC 4 W trap is 15.25 (12.7-18.3) m. Thus there is no overlap when the traps are placed more than 30.5 (25.4-36.6) m apart. The traps in the present study were placed with 50 m distance, and therefore the ranges of attraction did not overlap.

1750 A fourth concern is that the traps can compete with each other. We here define competition between traps as the effect on a trap catch introduced by the presence of another trap on the field: Specimens that are caught in one trap cannot be caught in another trap. Rigot et al. [111] investigated the competition between the more powerful Onderstepoort 8 W traps and found a statistical significant competition between the traps when placed 50 m apart, but not when placed 100 m apart. Competition between the CDC 4 W traps used in  
1755 this study will be lower than this and therefore we do not consider competition a problem in the present study. Furthermore, if there was important competition in the present study, it would likely have caused a depletion of specimens in the middle of the field, which we did not find (results not shown). The only way to avoid problems regarding competition would be to conduct a series of separate  
1760 and costly field experiments for each sampling scenario. Therefore we consider the present analysis a constructive shortcut to determine the optimal sampling procedure.

## B.5 Competing interests

The authors declare that they have no competing interests.

## 1765 B.6 Author's contributions

This project is a part of the PhD project by Carsten Kirkeby at the Veterinary Institute at the Technical University of Denmark. Carsten Kirkeby conceived the study, carried out the planning and the field work, the sampling analyses and wrote the manuscript. René Bødker participated in the planning of the field

1770 work, analysis and discussion of the results. Anders Stockmarr participated in  
the planning of the field work, and took part in the analysis and the discussion  
of the results. Peter Lind participated in the analysis and discussion of the  
results.

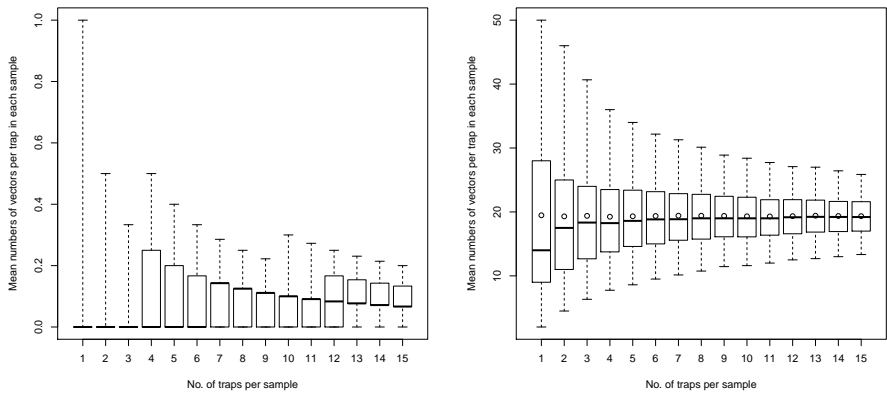
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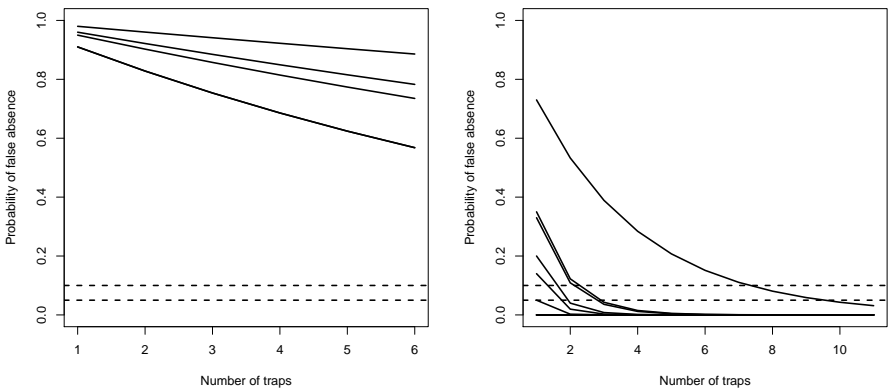
B.8 Figures

Figure 1



Bootstrap analysis of mean catches on two selected catch nights: Left: July 20<sup>th</sup>, right: July 21<sup>st</sup>. Circles illustrate the mean of 10,000 random samples, horizontal lines the median and whiskers show the 95% simulation envelope. Boxes show the 25% and 75% percentiles. In a) the pattern is inconsistent due to the low catch numbers. Increasing the number of traps in b) decreases the variation in the mean trap catch.

Figure 2



From the field data: The probability of falsely detecting absence on the field as a function of the number of traps used for sampling. Left: Six catch nights with more than 50% zero-catches. Right: Ten catch nights with less than 50% zero-catches. On five catch nights there were no probability of falsely detecting absence. Dotted lines show the 5% and 10% probability of falsely detecting absence of vectors. On catch nights with low vector abundance including more traps does not change the probability much. Few traps are needed to reach a low probability of false absence on catch nights with relative high abundance.

B.9 Tables

Table 1. Descriptive statistics for each catch night: The number of Obsoletus group specimens caught, the mean catch per trap, the number of analyzed traps, the precentage of zero-catches, the minimum catch and the maximum catch.

Date	20/7	21/7	27/7	3/8	4/8	6/8	17/8	18/8	21/8	24/8	25/8	27/8	28/8	31/8	03/9	04/9
Caught	4	872	316	173	522	612	2	93	95	29	427	1086	1	253	2	1
Mean	0.08	19.38	14.36	6.92	11.86	13.91	0.08	2.02	4.52	1.31	18.56	72.40	0.04	5.62	0.04	0.02
Traps	45	45	22	25	44	44	23	46	21	22	23	15	23	45	42	45
% Zero-catches	91	0	5	20	0	0	91	35	14	73	0	0	96	33	95	98
Min.	0	2	0	0	1	2	0	0	0	0	2	24	0	0	0	0
Max.	1	79	68	106	79	48	1	20	18	12	58	176	1	44	1	1

Table 2. Mean probabilities for a false negative result, depending of the number of traps. \* The modified dataset represents the data without five catch nights with more than 90% zero-catches.

Traps	1	2	3	4	5	6	7	8	9	10
Whole dataset	0.42	0.34	0.30	0.28	0.26	0.24	0.22	0.21	0.19	0.16
Modified dataset*	0.16	0.06	0.03	0.02	0.01	0.01	0.00	0.00	0.00	0.00

Table 3. The number of traps needed to reach a certainty of 90% or 95% of excluding a false negative result when sampling one, two or three nights. With the modified dataset (i.e. without low catch nights) a higher probability level is reached much quicker. \* The modified dataset represents the data without five catch nights with more than 90% zero-catches.

Full dataset			
Probability / Nights	1	2	3
90%	18	3	1
95%	25	7	2
Modified dataset*			
Probability / Nights	1	2	3
90%	2	1	1
95%	3	1	1

APPENDIX C

**The range of attraction for  
light traps catching *Culicoides*  
biting midges**

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## The range of attraction for light traps catching *Culicoides* biting midges (Diptera: Ceratopogonidae)

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Lasse E. Christiansen and René Bødker

### Abstract

**Background:** *Culicoides* are vectors of e.g. bluetongue virus and Schmallenberg virus in northern Europe. Light trapping is an important tool for detecting the presence and quantifying the abundance of vectors in the field. Until now, few studies have investigated the range of attraction of light traps. Here we test a previously described mathematical model (Model I) and two novel models for the attraction of vectors to light traps (Model II and III). In Model I, *Culicoides* fly to the nearest trap from within a fixed range of attraction. In Model II *Culicoides* fly towards areas with greater light intensity, and in Model III *Culicoides* evaluate light sources in the field of view and fly towards the strongest. Model II and III incorporated the directionally dependent light field created around light traps with fluorescent light tubes. All three models were fitted to light trap collections obtained from two novel experimental setups in the field where traps were placed in different configurations.

**Results:** Results showed that overlapping ranges of attraction of neighboring traps extended the shared range of attraction. Model I did not fit data from any of the experimental setups. Model II could only fit data from one of the setups, while Model III fitted data from both experimental setups.

**Conclusions:** The model with the best fit, Model III, indicates that *Culicoides* continuously evaluate the light source direction and intensity. The maximum range of attraction of a single 4W CDC light trap was estimated to be approximately 15.25 meters. The attraction towards light traps is different from the attraction to host animals and thus light trap catches may not represent the vector species and numbers attracted to hosts.

**Keywords:** *Culicoides*, range of attraction, vector abundance, light traps, vector monitoring.

## C.1 Introduction

Biting midges (Diptera: Ceratopogonidae: *Culicoides*) are vectors of e.g. Blue-tongue virus [25] and the newly discovered Schmallenberg virus in northern Europe [108]. Due to their crepuscular activity pattern, the standard trapping method is by (UV) light traps [130]. The vision of *Culicoides* and Ceratopogonids in general has not been studied well [3], although their phototactic behavior is of epidemiological importance. This behavior influences their response to light traps, which are widely used for determining the presence, abundance and phenology of *Culicoides* (e.g. [106, 34, 123]).

An optimal sampling strategy to estimate insect abundance must rely on knowledge of the area or range covered by a single trap. Many different terms have been used for this measure, and we prefer to use the 'range of attraction', describing the (maximum) distance at which insects are attracted to the trap. This term allows for a non-symmetrical attraction range around the trap which is relevant for traps equipped with light tubes. Only few studies have attempted to estimate the range of attraction for light traps: Odetoyinbo [101] made a study where a trap was hung at different distances from an open window which mosquitoes passed through at night. The aim was to estimate the point where the trap caught more than a simultaneously operated independent trap. Here, the 'effective range' for a CDC mini light trap was estimated to be approximately 5m. Baker & Sadovy [6] put up 125W mercury vapor lamps at different distances from release point of marked moths. By varying the distance from the release point to the lamps, they found that the response distance was within 3m. Lately, Truxa & Fiedler (2012) [125] made a study where marked moths were released at different distances to a UV-light trap. The catches showed that the 'radius of attraction' was up to 40m in 5-min intervals. For *Culicoides*, Rigot & Gilbert (2012) [111] made a study where 8W Onderstepoort light traps competed with each other at different distances. Background fluctuations in the *Culicoides* abundance were monitored by an independent light trap. The analysis assumed a fixed radius of each trap, regardless of the distance to other traps. The 'effective trap radius' was found to be approximately 30 m when the traps were running for 30-min intervals. However, Venter et al. (2012) [131], only found an 'range of attraction' for *Culicoides* for the same trap type of between 2 and 4 meters. In that experiment, two traps were hung with varying distances to each other and the background fluctuations were monitored using an independent trap. The 'range of attraction' was then the distance at which the two traps began to catch less than the independent trap. Both of these last studies hypothesized that *Culicoides* are attracted to the nearest trap, and that the light from the trap is isotropic (uniform in all directions). In the present study we incorporate the directionally dependent (anisotropic) light created by the light tubes in the light traps into two novel models for the attraction of

*Culicoides* to light traps.

We test three different models for attraction of *Culicoides* to light traps: Model I where the range of attraction for each trap is isotropic and independent of the distance to adjacent traps and where *Culicoides* fly to the nearest trap; Model II where *Culicoides* fly towards the direction with highest light-intensity, simulating anisotropic light and where overlapping ranges of attraction create an extended range of attraction; and Model III where overlapping ranges of attraction also create an extended range of attraction but where *Culicoides* continuously evaluate each light source in its field of view and fly towards the highest light-intensity. We use two different experimental setups to test the models. By fitting the models to the relative trap catches in the experimental setups we exclude factors influencing the level of abundance. The range of attraction for *Culicoides* is then estimated from the model that best fit the trap data in both experimental setups.

## C.2 Methods and Materials

Experiments were conducted in the summer of 2011, on a farm with approximately 70 cows in Klippinge, Denmark (geographical coordinates: N55.3619, E12.3234). The study field, measuring approximately 120 by 120 meters, was grazed by cattle during the day. Before dusk, the cattle were excluded from the field, but had access to enclosures on the western side of the study site. The surrounding land cover was grazed fields and grain fields, so there were no obstructions of *Culicoides* vision or flight next to the setup. Approximately 100 meters from the experimental setup there was a cow stable and a dunghill with potential breeding sites for the *Obsoletus* group. In a radius of 500 m, there were at least three ponds with potential breeding sites of the *Pulicaris* group. However, no breeding sites were monitored during the study period. The experiment was set up close to the cattle to ensure a high density of *Culicoides*. There were no other sources of light pollution present on the field during the experiments. *Culicoides* were caught using CDC 1212 mini light traps ([www.johnwhock.com](http://www.johnwhock.com)). These traps are equipped with a horizontally mounted 11 cm fluorescent tube emitting anisotropic UV-light. This means that the highest light-intensity is seen perpendicular to the tube and no light is seen from the ends of the tube. The light tubes were placed at a height of 180 cm and all light tubes were aligned along the transect line. Before each catch night, freshly charged batteries were installed on the traps. The starting time of sampling was decided each catch night to be when it was dark enough to perceive the light from the traps with the naked eye on a few meters distance. Traps were allowed to catch in intervals of one hour before they were emptied. Catch nights were chosen subjectively

for optimal flight conditions for *Culicoides*: low wind speed; no precipitation;  
 1940 high air humidity; no fog in the air; and not too low temperature. Weather  
 variables were monitored during the experiment using a Davis Vantage Pro 2  
 weather station.

### C.2.1 Experimental setup

**Experimental setup A** In the first experimental setup, 10 traps were set  
 1945 up in each of two transects with higher trap density towards the middle of  
 the transects, aligned east-west (fig. 1). Within each transect, the traps were  
 positioned at 0, 3, 9, 21 and 45 meters from the middle of the transect. In this  
 way, the distance between traps was doubled for each transect position. In the  
 middle position, two traps were placed with the light tubes separated by 12 cm.  
 1950 Two parallel transects were set up in each catch interval and separated by 100  
 meters. Setup A was run on the 27th (between 22.15 and 00.15 hours), 30th  
 (21.15-00.15 hours) and 31st (22.15-00.15 hours) of July 2011.

**Experimental setup B** In the second experimental setup, 6 traps were placed  
 1955 in two transects with higher density towards one end, also separated by 100  
 meters. This setup was either aligned north-east to south-west (E-W) or north-  
 easts to south-west (N-S) (fig. 1) in each catch interval. Within each transect, the  
 traps were placed at 0, 3, 9, 21, 45 and 93 meters from the starting point, also  
 doubling the distance between traps for each position. The transect directions  
 were reversed so the end with more traps pointed in opposite directions. This  
 1960 setup was run on the 17th (N-S, 21.45-00.45 hours), the 18th (N-S, 21.45-00.45  
 hours) and on the 24th (E-W, 21.15-00.15 hours) of July 2011.

All light traps were equipped with new light tubes and were aligned so all the  
 tubes were parallel to the transect direction. Insects caught in the traps were  
 sorted in a dissection microscope. *Culicoides* were identified by wing morphol-  
 1965 ogy according to Campbell & Pelham-Clinton [22] and female specimens of the  
*Culicoides obsoletus* group and the *C. pulicaris* group were identified and used  
 for analysis. The two species groups contributed separately to the dataset so  
 that the total number of study units were initially 54 transects, consisting of  
 different species groups, hourly catch intervals and transect positions.

### 1970 C.2.2 Models

We investigated three mathematical models to explain the observed fraction of  
 catch per trap per transect. Model I, 'Nearest trap', assumed a constant trap



radius where vectors always fly to the nearest trap, as suggested by Rigot & Gilbert (2012) [111]. Model II, 'Indirect light', calculates the combined light field surrounding the traps, then assumes that *Culicoides* always fly toward areas of higher light intensity, and in this way range of attraction becomes defined by a cutoff value in total light intensity. Model III, 'Perceived light', assumes that *Culicoides* fly in the direction of what they perceive as the brightest light source. The model approximates the perception of a light trap for *Culicoides* with a Gaussian function, which means that the lights from closely placed traps overlap. Range of attraction is again defined by a cutoff in light intensity.

**Model I - Nearest trap:** Effectively this model states that *Culicoides* always fly towards the nearest trap. Consequently traps are assumed to catch a number of *Culicoides* proportional to the area within the range of attraction,  $r$  (IA/B in fig. 2). If the distance,  $d$ , between two neighboring traps is smaller than two times the range of attraction, each trap's area of catch is reduced by half of the overlapping area. The model only allows for the catch area to be reduced by the nearest neighbor(s). The equations we present are only valid for traps on a line. The predicted fraction of catch,  $C_i^I$ , for the  $i$ 'th trap is then:

$$\begin{aligned}
 A_i^I &= \pi r^2 - \sum_{j \in NN} \left( 2r^2 \arctan \left( \frac{2\Omega}{d_{ij}} \right) - d_{ij}\Omega \right) \\
 \Omega &= \sqrt{r^2 - \left( \frac{d_{ij}}{2} \right)^2} \\
 C_i^I &= \frac{A_i^I}{\sum_{i=1}^n A_i^I}
 \end{aligned} \tag{C.1}$$

Where  $A_i^I$  is the area within range of attraction for the  $i$ 'th trap minus half the area of the  $j$ 'th trap, which is the one or two next neighbors,  $NN$ ,  $d_{ij}$  is the distance between the  $i$ 'th and  $j$ 'th trap, and  $n$  is the total number of traps in the transect.  $A_i^I$  is represented by one color per trap ( $i$ ) within the white lines (range of attraction) in fig. 2. This type of model was investigated for *Culicoides* by Rigot & Gilbert (2012) [111].

**Model II - Indirect light:** In this model we assume that *Culicoides* always fly toward areas of higher light intensity, quite similar to moving towards higher concentrations of scent molecules, when searching by smell. We therefore calculate the total light intensity,  $I(x, y)$ , for the area around the traps:

$$I(x, y) = \sum_{i=1}^n \frac{\Psi(\phi_i(x, y))}{(x - x_i)^2 + (y - y_i)^2} \tag{C.2}$$

$$\Psi(\phi_i(x, y)) = |\cos(\phi_i(x, y))| \tag{C.3}$$

Where  $n$  is the number of traps and  $x_i, y_i$  are the spatial coordinates of the  $i$ 'th trap. *Culicoides* in any given position are then assumed to fly along the highest gradient of  $I(x, y)$ . We model the anisotropic light field around traps using  $\Psi$ , which is a function of the angle around each trap,  $\phi_i(x, y)$ , in concordance with Lambert's cosine law. To model the attraction area we start one *Culicoides* for every square meter within at least 150 meters from the central trap, and simulate the attraction of light by individually moving them in small steps along the largest gradient in the surrounding light field until they eventually arrive at a trap location. From this we can determine a function  $T^II(x, y)$  which tells which trap a single *Culicoides* will fly to as a function of initial position (IIA/B in fig. 2). We can then define a cutoff,  $I_C$ , in light intensity that defines how far away *Culicoides* are attracted towards the light. And from this determine the fraction of catch of *Culicoides* for the  $i$ 'th trap,  $C_i^{II}$ :

$$C_i^{II} = \frac{\sum_{x,y} \mathbf{I}(I(x, y) > I_C \wedge T^II(x, y) = i)}{\sum_{x,y} \mathbf{I}(I(x, y) > I_C)} \quad (\text{C.4})$$

Where  $\mathbf{I}$  is the indicator function. This equation and figure 2 is then interpreted as that each trap in the transect catch a fraction of *Culicoides* proportional to the area within the light cutoff.

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**Model III - Perceived light:** This model aims to recreate the view that *Culicoides* have of the traps from every point on the area around the traps. *Culicoides* will fly in the direction of the perceived brightest light. For every point on the field a 360° view is generated with a resolution of 1°,  $V(x, y, \phi)$ . This is calculated by combining the light intensity from the  $i$ 'th trap at every point,  $I_i(x, y)$ . Combined with anisotropic light,  $\Psi$ , and the inaccuracy *Culicoides* perceive the position of the light traps,  $\sigma$ .

2010

$$I_i(x, y) = \frac{\Psi(\phi_i(x, y))}{(x - x_i)^2 + (y - y_i)^2} \quad (\text{C.5})$$

$$V(x, y, \phi) = \sum_{i=1}^n \frac{I_i(x, y)}{\sigma\sqrt{2\pi}} \exp\left(-\frac{(\phi - \phi_i(x, y))^2}{2\sigma^2}\right) \quad (\text{C.6})$$

We again start one *Culicoides* for every square meter and simulate their flight towards what they perceive as the strongest light until they arrive at a trap location. From this the function  $T^{III}(x, y)$  is determined which describes which trap a single *Culicoides* will fly to as a function of initial position (fig. 2). We define a cutoff,  $I_C$ , in light intensity that defines how far away *Culicoides* are attracted towards the light. And from this we determine the fractional catch of *Culicoides* per transect:

$$C_i^{III} = \frac{\sum_{x,y} \mathbf{I}(\max(V(x, y, \phi)) > I_C \wedge T^{III}(x, y) = i)}{\sum_{x,y} \mathbf{I}(\max(V(x, y, \phi)) > I_C)} \quad (\text{C.7})$$

The *Culicoides* fly in the direction they perceive to have the brightest light. When *Culicoides* are far away the trap lights seem to blend together because the angular distance between them is smaller and the  $\sigma$  smears the view. As illustrated in fig. 3.

**Range of attraction,**  $r^k$ , for the  $k$ 'th model must, in case of model II and III, be calculated from the light intensity cutoff,  $I_C^k$ , which is a result from the best fit of models to data. In the experimental setups the combination of light from the traps provide a complex pattern for the combined range of attraction as seen in figure 2. But for a single trap  $I_C^k$  determines the range of attraction perpendicular to the tube as:

$$\begin{aligned} r^k &= \sqrt{\frac{\kappa^k}{I_C^k}} \\ \kappa^{II} &= 1, \quad \kappa^{III} = \frac{1}{\sigma\sqrt{2\pi}} \end{aligned} \quad (C.8)$$

With  $\kappa^k$  being the light intensity one meter from a trap in the  $k$ 'th model. Model II and III are normalized differently because the total light intensity from one lamp across the view function,  $V$ , is set to sum to one in model III.

**Fitting to data:** To determine best fit we used the value of a  $\chi^2$ -test statistic ( $CS^k$ ) to evaluate the modeled fraction of catch  $C_i^k$ , from the  $k$ 'th model, with the observed data,  $C_{i,j}$ .

$$\begin{aligned} CS^k &= \sum_{i=1}^n \sum_{j=1}^m \frac{(C_{i,j} - E_{i,j}^k)^2}{E_{i,j}^k} \\ E_{i,j}^k &= C_i^k(r^k, \sigma) \times \hat{I}_j \end{aligned} \quad (C.9)$$

Where  $n$  is the number of traps,  $m$  is the catch number with  $\hat{I}_j$  being an identity vector of length  $j$ , so  $E_{i,j}^k$  is the expected fraction of catch from model  $k$  repeated  $j$  times equal to the number of separate catches. And  $C_i^k$  is the fractional catch from the models, which is dependent on range of attraction and also  $\sigma$  in model III. The best fit is the set of parameters  $(r^k, \sigma)$  that minimizes the value of  $CS^k$ .

This method puts equal weight on each transect of catch. Given that the  $C_{i,j}$  is the relative catch per trap per transect, the abundance of *Culicoides* does not have any impact on the analysis. This removes the need to include factors which affect abundance in the model.

Not all data was included in the trap data. A transect of trap data was omitted if there were more than three zero catches, which was usually observed on days with a very low total catch. Many zero catches would bias the catch distribution towards equal catches between traps, which would not represent the true catch

distribution, but merely reflect the variation in daily catch. In total 14 of 28 trap data sets for setup A were excluded, while only 5 of 26 were omitted for setup B. Thus a total of 35 transects were included in the analysis. The analysed data from setup A was consequently from 27th (between 23.15 and 00.15 hours), 30th (23.15-00.15 hours) and 31st (22.15-00.15 hours) of July 2011. For setup B analysed data was from 17th (N-S, 23.45-00.45 hours), the 18th (N-S, 21.45-00.45 hours) and on the 24th (E-W, 22.15-00.15 hours) of July 2011.

In the data there were three missing data points (NAs) for setup A and two NAs for setup B. These were handled by using an Expectation Maximization (EM) procedure [84]. The EM converged in all cases after maximum one step.

In setup A data is presented symmetrized by averaging over traps in pairs around the center of one transect of traps. We symmetrized data to remove directional bias in the experimental setup. However, the symmetrizing did not affect the fitting with the CS function, and symmetrized and un-symmetrized data gave the same results. We chose to present it symmetrized to allow for a better visual comparison with the models, which will always give a symmetrical result for setup A.

Confidence intervals on  $r$  and  $\sigma$  in model III were determined using Fischer information theory as presented in Madsen (2008) [86]. The method was implemented by approximating the CS-test curve to a second order polynomial using a power transformation to symmetrize around the minimum value of the CS function (9). Notice that this method produce non-symmetric confidence intervals.

To ensure that the model was not driven by the catch on one night, one experimental type of setup, or one *Culicoides* species we used the jackknife method on the data. Which is to reanalyze the data excluding the data from one catch night at the time, each species groups, or each experimental setup at the time.

## C.3 Results

10,150 *Culicoides* were caught and included in the analysis, of which 1,817 specimens were from the *C. obsoletus* group and 8,333 specimens from the *C. pulicaris* group. The hourly catches from each transect ranged between 3.6-27.8 (mean: 13.0) specimens per trap for the *C. obsoletus* group and 2.8-177.8 (mean:52.5) specimens per trap for the *C. pulicaris* group. Each transect in setup A comprised 90-278 (mean: 179.1) specimens from the *C. obsoletus* group and 312-1778 (mean: 970.3) specimens from the *C. pulicaris* group. In setup B,

each transect comprised 22-144 (mean: 61.8) specimens from the *C. obsoletus* group and 17-112 (mean: 63.4) specimens from the *C. pulicaris* group.

The catch nights were chosen subjectively for optimal flight conditions for *Culicoides*. During the catches, the temperature was between 12.9 and 18.6 degrees Celcius. The dew point temperature was below the ambient temperature during the whole study, and the air humidity was between 72% and 94%. The wind speed was between 0 and 1.8 m/s, and no precipitation was measured. Thus there was no rain, no fog in the air, high humidity, low wind speed and not too low temperatures.

The best fit of the models was determined by minimizing (C.9) as a function of the range of attraction  $r$  for model I, as a function of light intensity cutoff  $I_C$  in model II, and as a function of light intensity cutoff  $I_C$  and  $\sigma$  in model III (fig. 4). For model II and III  $I_C$  is recalculated to  $r$  by using (C.8).

The catch distribution of different ranges of attraction and the best fits of the models are presented in fig. 5. The collected trap data are numbered as presented in fig. 1. from left to right at  $y = 0$ . We generally observe that traps catch a lower fraction of *Culicoides* when placed closer together. However there is two very clear exceptions when traps are placed close together. The two central traps in setup A (number 5 and 6) catch almost the same as the outermost traps, and trap 1 in setup B catch a higher fraction than the other traps. These observations are strong indications that closely placed traps do not only compete for *Culicoides*, but also amplify attraction. The characteristics of the models compared to data are as follow (as seen in fig. 5).

Model I - Nearest trap: When range of attraction is lower than half the distance between the closest traps, the traps do not compete and will catch the same fraction of *Culicoides*. When going towards higher trap radius the center traps in setup A will catch a lower and lower fraction due to competition with neighboring traps. The outermost traps will always catch the highest fraction due to the lowest competition. Model I is therefore unable to reproduce the remarkable peak in the middle traps which is observed in data from experimental setup A. Moreover it overestimates the catch in the outermost traps in setup B.

Model II - Indirect light: In this model *Culicoides* are attracted towards higher total light intensity, which is a very simple way of considering attraction to light. Flying towards the strongest concentration of light attracts more *Culicoides* to the central area of the trap setups. This explains that for setup B the model predicts that traps 2 and 3 for medium and large ranges of attraction catch more than the outermost traps, which is contrary to the observed data. We therefore observe that the fitted  $r^{II}$  is very different whether fitted to setup A or B.

Model III - Perceived light: *Culicoides* in this model will fly directly towards the perceived brightest light source. The two central traps in setup A are located within such a small distance that *Culicoides* cannot distinguish them before  
 2120 within a very short distance, therefore they will appear as one trap with twice the brightness (fig. 3). This gives the added attraction to the middle traps which produce the central spike in the trap catch distribution which is also observed in data from setup A. Model III also fits data setup B where competition among traps 2, 3, and 4 reduce their fraction of catch compared to the outermost traps.  
 2125 Moreover trap 1 is predicted to catch a higher fraction due to the nearness of trap 2.

In model III the CS function for both setup A and setup B displayed a global minimum at  $\sigma = 10$  (left in fig. 4). With a combined 95% confidence interval 7.2-13.8. For  $\sigma = 10$  the CS is a continuous function of  $r$  with a unique global  
 2130 minimum at  $r^{III} = 15.25$  meters for both setup A and B (middle in fig. 4). With a 95% confidence interval 12.7-18.3. Furthermore we observe that for a broad range of  $\sigma$ s (also covering the 95% confidence interval) we observe that the optimal single range of attraction is approximately the same for setup A and B (right in fig. 4). Even though  $r^{III}$  is reported to 15.25 meters please  
 2135 observe that CS values were only calculated per one quarter of a meter, and the precision is not 1 centimeter.

The jackknife tests indicated that the range of attraction did not change significantly when excluding any of the catch nights, experimental setups, or species groups. In model III the only significant aberration of the value of  $\sigma$  was when  
 2140 excluding the catches on 30.07.11 the estimate changed to 14 with a 95% confidence interval 9.9-19.7, while all other results were within confidence levels (data not shown).

We notice that model I cannot fit any of the data (fig. 5, top). Model II can only fit data with very different values for  $r$  (fig. 5, middle). While model III  
 2145 is able to fit both setup A and B using the same values for  $\sigma$  and  $r$  (fig. 5, bottom). Since Model III is the only model which can fit both experimental setups with the same values of  $r$  we have not included a comparison of models using information criteria.

## C.4 Discussion

2150 In this study we found a range of attraction at 15.25m. This means that the trap type used in this study should be separated by at least 30.5m (25.4-36.6) to sample independently. When traps are placed closer than this, they will

influence each other, competing for *Culicoides*. However, the range of attraction will also be extended when catch areas overlap, which is a novel result of this study. Thus, it is possible to cover a greater area from the same position by using more than one trap.

We used the relative levels of catches to estimate the range of attraction. This made the modelling independent of weather parameters causing changes in the abundance, e.g. wind speed or temperature. Spatial parameters, e.g. wind direction and location of hosts are likely to have an impact on the relative catches in the traps. But we made an effort to compensate for this in the symmetrical shape of setup A, reversing the transects in setup B and by rotating setup B (fig. 1).

The range of attraction may differ between species. But Rigot et al. (2012) [111] found very similar ranges of attraction between vector species and vector species groups with overlapping confidence intervals. Because some species (e.g. *C. impunctatus*) may not be attracted by light as much as others [72], the range of attraction may be different for different species. Therefore we conducted a jackknife test on the result by removing a species group one at a time, which showed that the result did not differ significantly when testing the species groups individually.

Trap efficiency is dependent on the background illumination, which can differ between sampling periods due to factors such as cloudiness, moon phase and time of sampling related to sunset (e.g. [19, 16, 91, 100, 119]). This is a potential source of bias and could result in different ranges of attraction between sampling periods. But we tested this in a jackknife analysis, leaving out one catch night at a time, and found no significant difference in the estimate of the range of attraction. The background illumination is more most likely also impact on the  $\sigma$  parameter in model III because we expect that the *Culicoides* can distinguish light sources better under darker conditions. As previously stated, leaving out one of the catch nights did yield a significantly different estimate of  $\sigma$  (data not shown).

Model I (Nearest trap) failed to fit the data in experimental setup A and B. This model has a fixed range of attraction for each trap regardless of the distance to neighboring traps. This model type was used in the study of Bidlingmayer & Hem (1980) [16] to explain catch patterns of mosquitoes in traps without light, and it was recently used in another study to fit catches of *Culicoides* in light traps [111]. Given the physical properties of light, the effect of two neighboring light sources create an additive effect in the overlapping area, a main assumption in model II and model III. Thus we can see from this study that the range of attraction from one point can be extended by using more traps, corresponding to a stronger light source.

Model III (Perceived light) was the only model to fit both experimental setup A and B (fig. 5). Thus we regard 15.25m (12.7m-18.3m) as a reliable estimate of the range of attraction for one trap for *Culicoides* vectors. Rigot & Gilbert (2012) [111] found that the 8W Onderstepoort type traps had a range of attraction of 29.6 (26.3m-31.9m). However, since the model used in that study failed to fit both experimental setups in our study, a more precise estimate may be obtained by using Model III from the present study. The fact that Venter et al. (2012) [131] found a range of attraction between 2 and 4 meters for the Onderstepoort type trap, could indicate that other unknown factors may be important when traps are allowed to catch the whole night through.

As stated in [133], the range of attraction covers three concepts: the distance at which specimens can physically reach the trap within a given time interval; the distance at which a specimen can detect the trap; and the distance at which a specimen shows directed movement towards the trap. If traps are allowed to sample for longer time, data can be influenced by other parameters such as wind direction and wind plumes created by host animals. If sampling time is too short, the specimens within the range of attraction may not be able to reach the trap before the sampling ends. To investigate the range of attraction and the influence of time, different sampling intervals would be needed, which is worth further research.

The distance from the *Culicoides* to a trap is also worth considering. We assumed general random flight with full attraction towards the traps within the range of attraction. However, the traps might attract a higher percentage of *Culicoides* in the near vicinity of the traps compared to further out in the range of attraction, possibly proportional to light intensity.

In our models we assumed that *Culicoides* disperse evenly within the field. However, the abundance of *Culicoides* is likely to be higher near the cattle. We have tried to compensate for this by reversing the direction of the transects in setup B. Setup A compensate for this by the symmetry of the transect (fig. 1). Furthermore, the *Culicoides* may not be evenly dispersed when consecutive trapping is carried out because *Culicoides* within the range of attraction would be caught in the first trapping period and thus new *Culicoides* in the area would have to migrate in by random flight. To explain this pattern, a better fit may be obtained by fitting data to the circumference of the attraction area rather than the area itself. This could be worth investigating in future research. In both model II and model III, we assumed that all *Culicoides* caught in the traps approached the traps from the same height as the light sources, and therefore there were blind angles in the ends of the light tubes. If the *Culicoides* approached the traps from a lower height, the blind angle would be less pronounced. Although *Culicoides* have been caught at higher altitudes (e.g. [28]), and Venter et al. (2009) [129] caught most *Culicoides* at a height of 2.8m in South Africa, the



main flying height for *Culicoides* vectors in northern Europe is still unknown.

In Model II and III the light distribution around each trap was anisotropic. We also simulated these two models using isotropic light, but for both models anisotropic light fitted data better (data not shown). This indicates that the direction of the light tube in the trap is important, which has practical implications when catching *Culicoides*. If a certain area is to be monitored in a study, e.g. an enclosure with host animals, the catch size will depend on the angle of the trap to the area of interest. If trap catches are to be compared in a study, the standardization procedure should include direction of the light traps.

In this study, Model II modelled the light from each trap, resulting in higher light intensity when ranges of attraction overlap. This is comparable to a scent zone created isotropically around a host animal if there is no wind present. This model did not fit data as well as Model III did, where the *Culicoides* can perceive the individual sources of light at a distance and head for the strongest light source. This is an important biological finding and indicates that the *Culicoides* show directed movement towards a light source rather than a more random flight towards areas with higher light intensity. The implications of this finding is important for other studies using light trap catches to estimate the number of *Culicoides* (and possibly also other insects, e.g. mosquitoes) attracted to host animals. We have here shown that the vectors evaluate light sources at a distance. This behaviour is different from how we assume the vectors are attracted to host animals, i.e. following a plume of scent. Thus light trap catches may not be representing the number and species of vectors attracted to hosts very well, and should be used with caution.

## C.5 Conclusions

We tested three different models to fit two different field data sets, and showed that the *Culicoides* are likely to locate the light by evaluating the direction of the strongest light source in their field of view and then fly towards it rather than flying towards the nearest trap. We estimated the range of attraction for a single CDC 4W UV light trap to be 15.25m (12.7-18.3) perpendicular to the light tube. Therefore we suggest that, in future studies, traps of this type are separated by at least 30.5m (25.4-36.6) in order to be independent. If they are placed closer than this, their interactions should be modelled as in model III in this study. Light traps may not represent the number of vectors attracted to hosts because the attraction behaviours are different.

## C.6 Competing interest

2270 The authors declare that they have no competing interests.

## C.7 Author's contributions

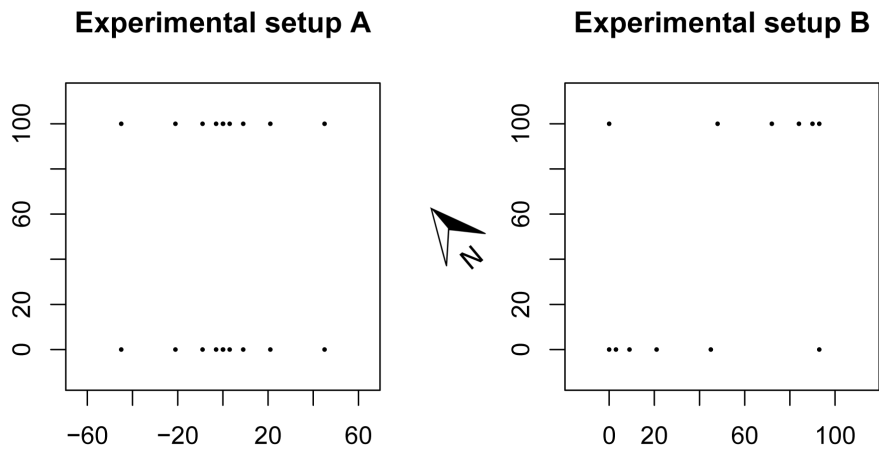
2275 This project is a part of the PhD project by Carsten Kirkeby at the Veterinary Institute at the Technical University of Denmark and the PhD project by Kaare Græsbøll at the Veterinary Institute and Department for Informatics and Mathematical Modelling, Technical University of Denmark. Carsten Kirkeby conceived the study, carried out the field work, participated in discussion of the modeling procedure and wrote the background, experimental setup, experimental setup results, discussion and conclusion sections of the manuscript and created fig. 1. Kaare Græsbøll conceived the framework of model II and III, carried out all modeling and model fitting and wrote the modeling and the model results sections of this paper. Kaare also participated in the discussion and conclusions sections and created figs. 2, 3, 4 & 5. René Bødker participated in conceiving the study, planning of field experiments and discussion of the results. Anders Stockmarr participated in the planning of the field work and model fitting and evaluation. Lasse E. Christensen participated in conceiving model III, the modeling procedure, model fitting and evaluation.

## C.8 Acknowledgements

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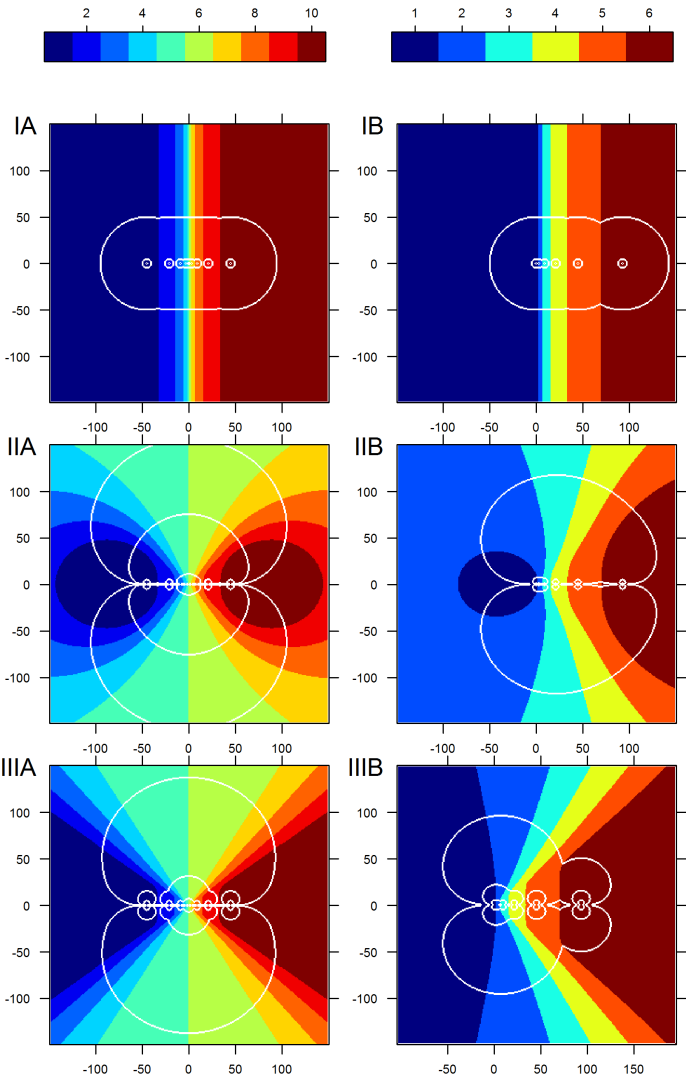
C.9 Figures

C.9.1 Figure 1 - Experimental setup



Experimental setup A and B. The plots shows the study area viewed from above. Distance units are meters. Traps are represented by black dots. All traps were hung with the light tubes along the transect line. In setup A, the middle dot represents two traps separated by 12 cm. In setup B, the configuration was rotated 90 degrees in some time intervals.

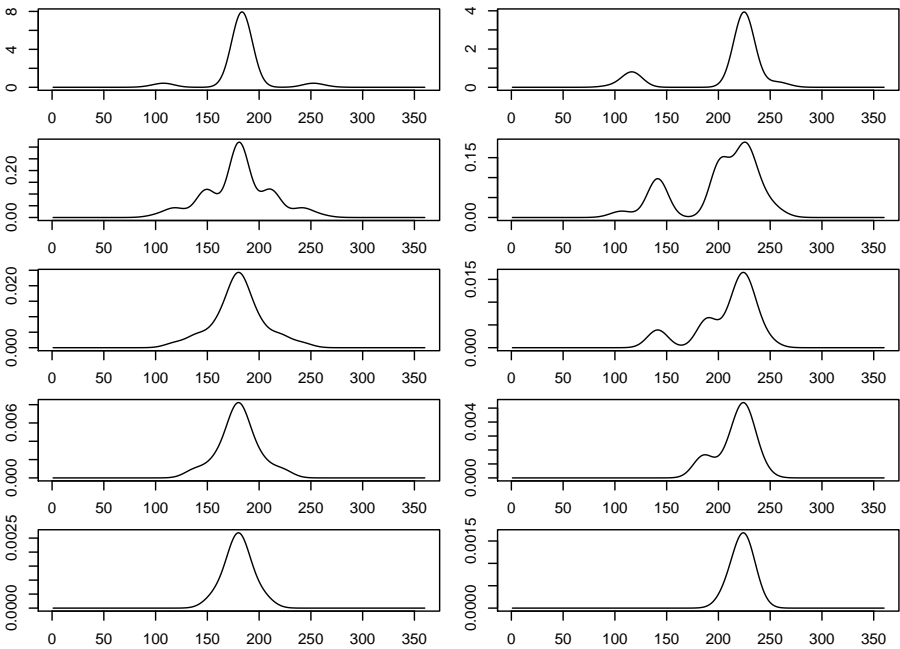
C.9.2 Figure 2 - Range of attraction vizualisation



2305 The area of attraction for the three models. It is assumed that each trap catch a number of *Culicoides* proportional to the area within the range of attraction (white lines). The plots represents modeled fields of 300 by 300 meters with one

transect of traps from fig. 1. The colors (color-key on top) indicate which trap a *Culicoides* in this area will end up in, with trap numbers corresponding to numbers in fig. 5. The white lines indicate different cutoff in range of attraction (light intensity). These plots thereby represent the functions  $T^k(x, y)$ , where the white lines in each plot indicate the three cutoffs,  $I_C$ , also indicated in fig. 5, which correspond to a range of attraction of 5 and 50 meters and the best fit. Model III is presented with  $\sigma = 10$ . The left column is setup A, the right column is setup B. In model I (top) *Culicoides* always fly to the nearest trap, in model II (middle) *Culicoides* fly towards the area of highest light intensity, and in model III (bottom) *Culicoides* fly towards what they perceive as the brightest light, as illustrated in fig. 3.

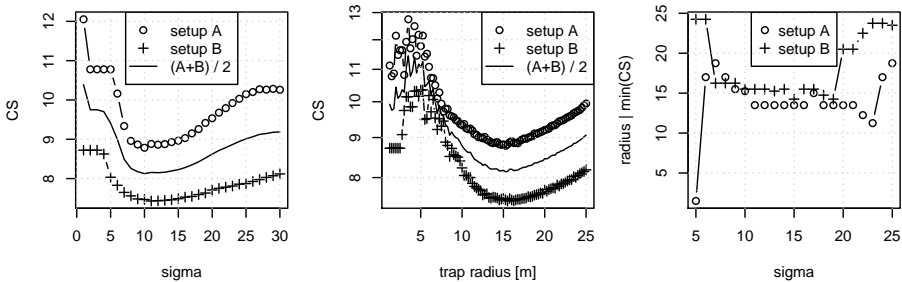
C.9.3 Figure 3 - 360 degrees view of a *Culicoides*



The light intensity of setup A as perceived by one *Culicoides* as a function of degrees on the angle of the transect. X-axes show the view angle in degrees where zero degrees is downwards orthogonal on the transect line and the angle increases counterclockwise. Plots show views as if a single *Culicoides* approaches the central traps in a straight line perpendicular to (left), or in a 45 degree

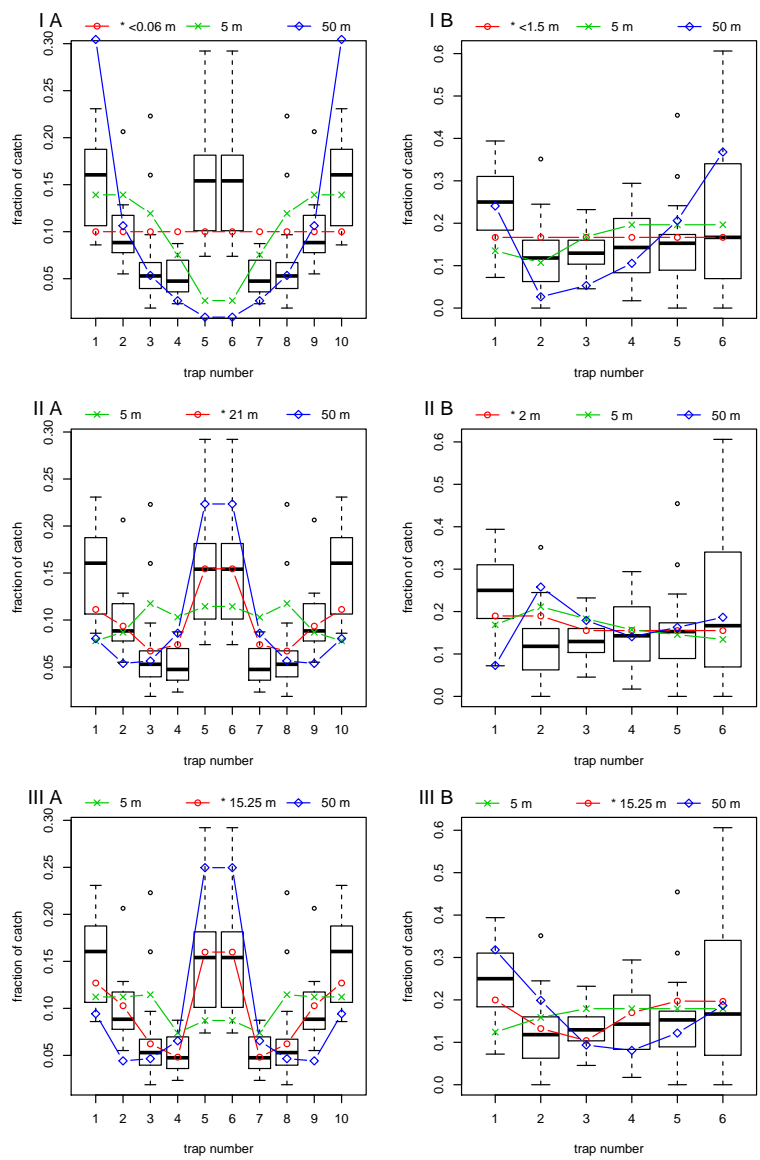
angle to the transect (right). In the coordinate system of fig. 2 and equation (C.6), this correspond to the coordinates  $(0, -i)$  (left) and  $(-i, -i)$  (right) where  $i = 1, 5, 25, 50, 100$  meters, with  $\sigma = 10$ . *Culicoides* are assumed to fly towards the brightest perceived light, and as the perception is assumed to be Gaussian, *Culicoides* must be close to the transect (distance depends on  $\sigma$ ) to differentiate neighboring traps.

C.9.4 Figure 4 - Model fits of sigma and range of attraction



CS values from eq. (C.9) as a function of  $\sigma$  (left), and range of attraction,  $r$ , with  $\sigma = 10$  (middle). The minimum CS value indicates which value best fits with the observed data, when using model III. Right is the range of attraction,  $r$ , that minimizes CS for different values of  $\sigma$ , which shows stable range of attraction for a range of  $\sigma$ . All plots are for model III. The jump in values around  $\sigma = 20$  in the rightmost figure is due to range of attraction exceeding the simulation box size. The noise for small trap radius and small  $\sigma$ s is due to rounding errors that occurs on small scales.

C.9.5 Figure 5 - Model fits to experimental setups



2345

Predicted fractional catch per trap given model I (nearest trap), II (indirect light), and III (perceived light) (top, middle, bottom) with the field data as boxplots, for setup A and B (left, right). The prediction is the fractional part

of the area covered within the range of attraction in fig. 2. The red line with  
 2350 circles is always the best fit of the model to the data. The green and blue line  
 is predicted fractional catch with a range of attraction of 5 or 50 meters. The  
 ranges of attraction  $r^k$  giving the best fit for the  $k$ 'th model were:  $r^{IA/B} <$   
 $0.06/1.5$  m,  $r^{IIA/B} = 21/ < 2$  m, and  $r^{III} = 15.25$  m with  $\sigma = 10$ .





APPENDIX D

2355

**Quantifying movements of  
European *Culicoides* vectors  
between farms using a novel  
mark-release-recapture  
technique**

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## Quantifying movements of European *Culicoides* (Diptera: Ceratopogonidae) vectors between farms using a novel mark-release-recapture technique

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### Abstract

Studying the dispersal of small flying insects such as *Culicoides* constitutes a great challenge due to huge population sizes and lack of a method to efficiently mark and objectively detect many specimens at a time. We here describe a novel mark-release-recapture method for *Culicoides* in the field using fluorescein isothiocyanate (FITC) as marking agent without anaesthesia. Using a plate scanner, this detection technique can be used to analyse thousands of individual *Culicoides* specimens per day at a reasonable cost. We marked and released 853 specimens of the Pulicaris group and 607 specimens of the Obsoletus group on a cattle farm in Denmark. An estimated 9,090 (8,918 - 9,260) Obsoletus group specimens and 14,272 (14,194 - 14,448) Pulicaris group specimens were captured in the surroundings and subsequently analysed. Two (0.3%) Obsoletus group specimens and 28 (4.6%) Pulicaris group specimens were recaptured. The two recaptured Obsoletus group specimens were caught at the release point on the night following release. Eight (29%) of the recaptured Pulicaris group specimens were caught at a pig farm 1,750 m upwind from the release point. Five of these were recaptured on the night following release and the three other were recaptured on the second night after release. This is the first time that movement of *Culicoides* vectors between farms in Europe has been directly quantified. The findings suggest an extensive and rapid exchange of disease vectors between farms. Rapid movement of vectors between neighboring farms may explain the the high rate of spatial spread of Schmallerberg and bluetongue virus in northern Europe.

**Keywords:** *Culicoides*, Schmallerberg, bluetongue, dispersal, movement, mark-release-recapture.

## D.1 Introduction

2390 Vectorborne diseases are of great concern in all parts of the world. In northern  
Europe, incoming disease agents such as bluetongue virus and Schmallenberg  
virus have recently appeared where *Culicoides* borne diseases have previously  
not been a problem (e.g. [89, 27]). Epidemiological models for the spread of  
vectorborne diseases such as bluetongue virus rely on accurate data describing  
2395 the underlying mechanisms [9, 60, 58]. Especially the dispersal distance, speed  
and direction is of high importance when simulating outbreaks of vectorborne  
diseases [51, 118, 58].

Mark-release-recapture (MRR) techniques have been used in many studies to  
investigate the behavior of different insects, e.g. beetles [126], grasshoppers [73],  
flies [93], termites [57], mosquitoes [68] and fruit flies [35]. In MRR studies, it  
2400 is necessary to mark a relatively large proportion of the population because the  
propability of recapture can be very low as a result of mortality and emigration.  
The number of *Culicoides* specimens at a location can be enormous in  
some places, reaching over a thousand specimens caught in a single trap [89].  
Thus MRR studies of *Culicoides* requires a high number of marked specimens  
2405 and high-throughput detection. It also requires a sensitive detection technique  
because of their small size.

Only very few MRR studies have been conducted on *Culicoides* previously:

In 1977, Lillie et al. [83] anesthetized, marked and released 82,200 specimens  
of *Culicoides variipennis* with micronized fluorescent dust in Denver, Colorado.  
2410 403 marked specimens were recaptured in CO<sub>2</sub>-baited traps. Recaptured spec-  
imens were detected by eye inspection under UV-light. They found one female  
that had dispersed 4 km in 36 hours.

Brenner et al. [20] studied *C. mohave* in the desert of Southern California in  
1981. Traps were baited with dry ice. In the marking procedure, specimens  
2415 were anaesthetized with CO<sub>2</sub> and shaken in a container with fluorescent pow-  
der. Marked specimens were detected by examination under UV-light on a black  
background. In that study, almost 14% of 20,646 marked specimens were recap-  
tured. They found that most specimens dispersed downwind but also found a  
female 6 km upwind 30 hours after release. They further speculated that *Culi-*  
2420 *coides* exhibit omnidirectional flight rather than either upwind or downwind  
dispersal, although most specimens in this study were caught downwind.

In 1984, Lillie et al. [82] conducted a study where 40,000 specimens of *Culicoides*  
*mississippiensis* were marked and released. In this study no anaesthetization  
was used and *Culicoides* were caught in CDC light traps baited with CO<sub>2</sub>.

During two-four day periods following two releases, 567 (1.4%) specimens were recaptured up to 3.2 km away from the release point. At this position a single specimen was caught 24 hours after release. There were no indications of influence of wind direction on the flight direction in this study.

According to Hagler & Jackson [64], an ideal marker for insects is "durable, inexpensive, nontoxic, easily applied, and clearly identifiable". Until now, MRR studies of *Culicoides* have been based on subjective visual eye inspection to detect marked specimens under UV light. Here we take a new approach and use a novel method for marking *Culicoides* with an objective method of detection of marked specimens.

Most models for the spread of bluetongue virus assume that vectors fly in random directions and can be transported with the wind over long distances. Recently, [118] developed a model to simulate the 2006 outbreak of BTV in northern Europe including upwind flight of the vectors. They found that downwind flight, as included in previous models, was not sufficient to explain the number of infected farms. Thus they included upwind flight and mixed random flight, and were able to explain 94% of all observed farm infections. They concluded that upwind flight of the vectors was responsible for 38% of the infections. In this study we directly quantify the dispersal of European *Culicoides* vectors between farms for the first time.

## D.2 Materials and Methods

### Marking method

Fluorescein is an orange staining dye commonly used in microscopy. If excited with fluorescent light at approx. 494 nm, it emits light at approx. 521 nm and is therefore a useful tool in ELISA plate scanning. Fluorescein isothiocyanate (FITC) is fluorescein with a reactive SCN group (thiocyanate), used previously to label chitinase [13]. FITC in powder form must be kept in a dark container in order not to fade, but is otherwise stable.

We used FITC powder in this study to mark the specimens. The amount of powder that can adhere to small specimens of *Culicoides* is of course small, making detection with the naked eye difficult. Therefore we used a Tecan SpectraFluor Plus plate scanner and the Xfluor software ([www.tecan.com](http://www.tecan.com)) for detection of FITC on specimens. To each well in ELISA plates with flat bottom were added 100  $\mu$ L 70% ethanol to extract the FITC and preserve the *Culicoides*. It also

removed most of the static electricity which could make it difficult to place the  
2460 dry specimens in the wells. All plates, with one specimen of *Culicoides* in each  
well, were gently shaken on a shaking table for five minutes prior to scanning.  
The plates were then scanned in the Tecan scanner with excitation wavelength  
set to 485 nm and emission wavelength set to 530 nm. Gain was set to 55 in all  
2465 trials and measurements were carried out with three flashes, 0 s lag time, 40  $\mu$ s  
integration time and an initial 10 s shake to distribute dissolved FITC in the  
ethanol. All plates were scanned twice, to increase the precision of detection.  
After scanning, the resulting data files were run through an automated procedure  
in R 2.14.2 (R Development Core Team, 2011), screening for measured  
values higher than a defined cutoff level.

2470 To identify a cutoff level for unmarked specimens, 192 *Culicoides* from the field  
experiment (see below), caught on the day before marking experiments started,  
were scanned twice using the scanning procedure. In order to exclude false positive  
specimens from the data, the cutoff was set to mean + 5\**st.dev.* Assuming  
a normal distribution and using this level, only one in 1.7 million specimens will  
2475 be false positive. At this cutoff level some marked specimens are likely to be  
undetected and wrongly classified as negative, but the priority in this study was  
to avoid any false positives because false negatives do not affect the proportional  
estimates of dispersal.

To validate the method we tested for cross-staining, laboratory contamination  
2480 and carryover of emitted light between wells. We marked dead specimens by  
shaking them in a beaker with FITC powder. They were then transferred to a  
clean beaker with unmarked dead specimens and shaken for one minute. To test  
for contamination from using the same tweezers to handle marked and unmarked  
insects, we placed ten lab marked specimens in a plate and subsequently used  
2485 the same tweezers to place six unmarked specimens.

There was a potential risk of a carryover effect of fluorescent light from a marked  
specimen in a well, to neighboring wells in the same plate with unmarked specimens,  
because regular transparent ELISA plates were used. To test this, dead  
specimens of *Culicoides* were marked by shaking them in a beaker with FITC.  
2490 Then five of these marked specimens were put into the wells on a plate with  
unmarked neighbors using the procedure as described above. The plate was  
then scanned as in the procedure described above.

### D.2.1 Field experiment

The field experiment was conducted between July 21 and August 14, 2010, on a  
2495 study farm in Denmark (geographical coordinates: N55.35477, E12.381). This

farm was chosen because the nearest farm was 1,750 m away which is a large distance in Denmark. The entire stable walls and the sliding doors in the ends were open, allowing *Culicoides* to freely enter and leave. The nearest farms were a small outdoor angus cattle holding with 20 outdoor animals at a distance of 2.0 km (West-North-West of the study farm) and a pig farm with about 1,700 animals indoors at a distance of 1.75 km (West of the study farm, see Fig. 1). The odor of pigs was emitted from the pig farm through a ventilation system. We also checked that no host animals that might attract *Culicoides* were present in other locations in the study area. During the study period a weather station (Davis Vantage Pro 2) measured the wind direction and temperature in 10 min intervals. The weather station was set up in the study area more than 100 m from any trees that could obstruct the wind. Supplementary data on the wind direction from an official weather station 10 km from the release point (Danish Meteorological Institute) was used in periods when the local weather station was not working.

Breeding sites for *Culicoides* were distributed throughout the study area. For the Obsoletus group, potential breeding sites were in leaf litter and decaying wood in forest areas primarily 400 m east of the farm, dung in the stables and a big dunghill next to the stables. Potential breeding sites for the Pulicaris group were present on surrounding fields around small ponds and marl pits [76, 45, 98].

Around the study farm, 45 traps were set up in locations approximating four transects out from the farm (see Fig. 1). On the pig farm 1,750 m west of the study farm (and release point), two groups of three traps each were hung up side by side near the stable, assuming that the abundance of *Culicoides* would be high here, and that *Culicoides* from the release points might disperse towards the pig farm. The trap type used was the CDC New Standard Miniature 4 W Blacklight Trap (Model 1212, [www.johnwhock.com](http://www.johnwhock.com)) using a 6 V battery and equipped with a photoswitch that automatically turned the trap on at dusk and off at dawn. Traps were hung up in a height of approximately 180 cm, on the stable wall, in branches on windbreaks where available and otherwise in heavy metal gallows constructed for the purpose. In each of three locations on the study farm, four traps were hung up side by side on the stable walls. At each of these three locations, trap catches were marked and released. The *Culicoides* were not anaesthetised upon marking, hence the number of marked specimens could not be counted directly. Therefore, to estimate the number of individuals marked and released, the specimens caught in the fourth trap was killed and preserved in 70% ethanol. We assumed that this trap caught 1/4 of the total catch in each location, which was the general pattern observed on the catch nights where all four traps were killed and analysed. On the 07. August, extra *Culicoides*, caught at a farm 3 km away (geographical coordinates: N55.3619, E12.3234), were released together with the other released specimens on the same day, in order to increase the number of marked specimens. The

number of released specimens from this location was estimated by another trap catching *Culicoides* side by side with the marked trap (Table 1).

2540 Before the study, a schedule was set up for marking specimens on the study  
farm once a week to allow marked specimens to disperse between markings.  
However, if low numbers of *Culicoides* were caught on the night planned for  
marking, it was postponed to the next night with catches high enough for feasible  
marking. We succeeded to mark *Culicoides* on four different dates during the  
2545 study period with minimum five days between markings. We marked specimens  
in the morning of the July 22<sup>nd</sup>, July 27<sup>th</sup>, August 1<sup>st</sup> and August 7<sup>th</sup>. The  
periods between markings and until August 14<sup>th</sup> after the last marking date are  
referred to as the marking periods (P1-P4 in Table 1).

The marking was carried out in the morning at the locations where the speci-  
2550 mens were caught, using the following procedure: A flow of air was created with  
a dust blower commonly used to clean camera lenses (InnoDesk, Inc., Cleveland,  
Ohio, USA). The dust blower runs on batteries so it can be used in the field,  
and creates a moderate consistent stream of air just enough to make a cloud of  
powder particles but not enough to kill the *Culicoides*. The air was led through  
2555 a 50 cm long and 0.6 mm wide plastic tube into a small (9 cm, 38 mm diameter)  
closed beaker containing approx. 5 ml FITC. In this beaker, the FITC powder  
was mixed with air into a dust cloud. From the beaker, the dust cloud was  
lead further through another 50 cm long and 0.6 mm wide plastic tube into a  
500 ml beaker with the caught insects. The plastic tube entered the beaker  
2560 through a hole in the lid, and the air stream escaped through another hole cov-  
ered with a fine mesh. The insects were gently swirled around in the flow of air  
for approx. 5 seconds, ensuring that all specimens had been in contact with the  
orange marking powder. After marking, insects were released onto the ground  
at the catch site. Plastic gloves were worn at all times when marking, and all  
2565 marking equipment was carefully packed separately from other equipment to  
avoid contamination.

All caught *Culicoides* that were not marked and released were killed quickly  
with a small piece of paper stained with ethyl acetate. They were then stored  
at -20 °C. Only a subsample of each trap catch was morphologically identified,  
2570 following Campbell & Pelham-Clinton [22]. If containing more than 20 speci-  
mens, catches were subsampled according to the Raosoft sample size calculator  
([www.raosoft.com/samplesize.html](http://www.raosoft.com/samplesize.html)) using 5% error margin and a confidence  
level of 95%. Females were then transferred to an ELISA plate with one speci-  
men per well. This allowed all specimens to be scanned individually. Each plate  
2575 was scanned twice in the Tecan scanner, and the mean value of the two scans  
was used as the measure of fluorescence. All positive specimens were identified  
to species group level.



## D.3 Results

### D.3.1 Method validation results

The fluorescence cutoff value between negative (unmarked) and positive (marked) specimens were defined as the mean of the negative controls, consisting of the mean of two scans, plus five times the standard deviation of those values. The mean value was 45, and the standard deviation 18.5, and thus the cutoff for negative measurements was 138 for the described scanning conditions. We used the mean value of two scans as a measure of fluorescence, which resulted in 30 specimens with a mean value higher than the cutoff. The correlation between the first and the second scan for the negative specimens was 0.65, and for the positive specimens 0.996.

The mean of the measured fluorescence emission of the laboratory marked specimens in the carryover study were approximately ten fold higher (minimum: 9,323) than the marked and recaptured specimens in the field (maximum: 1,701). The ranges of the scanned value of negative wells and the wells that were neighbours to a well with marked specimen overlapped and thus we did not test this further. No cross-staining between specimens or contamination from tweezers was detected (data not shown).

### D.3.2 Field study results

An estimated 607 *Obsoletus* group and 853 *Pulicaris* group specimens were marked and released, and an estimated 9,090 female *Obsoletus* group and 14,272 female *Pulicaris* group specimens were caught during the study period (Table 1). Of these, two (0.3%) of the marked *Obsoletus* group specimens and 28 (3.3%) of the marked *Pulicaris* group specimens were recaptured. This yields a total recapture percentage of 2.1%. The mean of fluorescence values of the marked specimens was 264, ranging from 142 to 1,701. The fluorescence values and recapture distance from the release point is shown in Fig. 2. The two recaptured *Obsoletus* group specimens were both caught in the first marking period where it was estimated that only 96 *Obsoletus* group specimens were marked (Table 1).

The two recaptured *Obsoletus* group specimens were caught in a trap at the release point for marked specimens. They were caught on the first night in the first marking period, meaning that they had been marked for maximum 24 h before recapture.

An overview of the results of the first release period is shown in Fig. 1. In the first marking period, 25 specimens of the *Pulicaris* group were recaptured out of an estimated 274 marked specimens. In the second release period only one *Pulicaris* group specimen was recaptured at the pig farm on the second night after release. In the third release period two *Pulicaris* group specimens were recaptured in the release point. In the fourth release period no marked specimens were recaptured.

In total, 18 of the *Pulicaris* specimens were recaptured on the first night after release; nine specimens were recaptured on the second night after release; and one specimen was recaptured four nights after release. Eight (29%) of the recaptured *Pulicaris* group specimens were caught on the pig farm at 1,750 m distance from the release points of marked specimens. Of these eight specimens, five (63%) were recaptured on the neighboring pig farm one day after release, having dispersed 1,750 m in less than 24 hours. The last three (38%) of the eight specimens were caught at the pig farm on the second night after release. From the *Pulicaris* group, 17 (61%) of the recaptured specimens were caught in the traps at release points of marked specimens. A single *Pulicaris* group specimen was recaptured after one night in a trap 250 m north-west of the release point; and two *Pulicaris* group specimens were caught on the second night after release, one in a trap 100 m north-west of the release point and the other one in a trap 1 km south of the release point (Fig. 1). During the whole study period the mean number of specimens caught per trap declined for both species groups, indicating that the abundance was declining (Table 1).

Because there exists no gold standard test that can be used to evaluate the cutoff, we also removed half of the specimens with the lowest half of the mean fluorescence values from the data. This was to test if the specimens caught on the pig farm had low fluorescence values. Using this high cut off, again 29% (4 out of 14) *Pulicaris* group specimens were recaptured on the pig farm. The fluorescence values are shown in Fig. 2.

Weather variables were measured during the whole study. All values presented are measured during the *Culicoides* active periods, which we defined to be one hour before to three hours after sunset and two hours before to one hour after sunrise. The wind direction was predominantly from west during all four study periods. In the first period the wind blew mostly from west and north-west; in the second period it blew from south-west; in the third period it blew from north-west; and in the fourth period it blew from south-west and north-west. The mean wind speed was declining during the four periods, going from 1.4 to 0.8 m/s (Table 1). Also the maximum wind speeds measured declined during the study period, going from 5.4 to 2.7 m/s. The mean temperature did not change much during the study period, but the minimum temperature in the *Culicoides* active periods went from 10.4 to 8.7 °C (Table 1).

## D.4 Discussion

We have here presented and tested a novel technique to mark and recapture *Culicoides* in the field and subsequently scan them individually. We have only used the technique for quantifying the proportion of marked specimens moving from one location to another. If the technique should be used for e.g. survival rate studies, more tests are needed, for instance how fast the light-sensitive FITC fades in nature. We have also not tested the impact of the marking method on the survival rate of marked specimens.

Most models for the spread of bluetongue assumes random local flight of the vectors [40, 67, 51, 58].

In this study we found that 29% of the recaptured *Pulicaris* specimens were recaptured at the pig farm, indicating that vectors actively disperse upwind to seek hosts like e.g. female host-seeking mosquitoes [50]. This is in contrast to the findings of Brenner et al. [20] who found that marked specimens of *C. mohave* dispersed omnidirectionally but mostly downwind. However, in that study a single female was recaptured 6 km upwind after 30 hours. Bhasin et al. [15] found that females of *C. impunctatus* showed upwind flight towards plumes of CO<sub>2</sub>. In the present study we found that 29% of the *Pulicaris* group specimens dispersed upwind. This supports the intense upwind dispersal, which Sedda et al. [118] found responsible for 54% of the infected farms in 2006. In that study, it was assumed that vectors could detect the odor of neighboring farms at a maximum distance of 300 m. Our results indicate that this distance is at least 1,750 m for the *Pulicaris* group. This is, to our knowledge, the first time that dispersal of European *Culicoides* vectors have been quantified between farms. The described measures of speed, distance and direction related to wind is useful when modeling the spread of e.g. bluetongue and Schmallenberg virus. However, we were not able to recapture more than two *Obsoletus* group specimens, the supposed main vector for BTV in northern Europe [27], and thus further studies are needed to investigate the dispersal pattern for this species group.

In 2008, 97.5% of the Danish cattle farms were placed within 1600 m distance of the nearest cattle farm (Kaare Græsbøll pers. comm.). Thus the results of this study suggest that vectors are capable of transmitting disease between almost all Danish farms very efficiently.

The sensitivity of the present technique is potentially higher than in previous studies [20, 82] because the scanning procedure used in this study can detect very small amounts of FITC. An advantage of the present technique is also that the insects can be marked without anaesthetisation, unlike some previous studies [20, 82]. By marking live specimens, mortality and morbidity of the

insects due to anaesthesia is avoided and their behavior is likely less interrupted. Furthermore, the detection of marked specimens in this study does not rely on subjective judgement of whether a specimen is marked or not.

When setting up field experiments for small flying insects such as *Culicoides*, weather conditions will influence the catch numbers greatly [114, 77]. The more specimens that are marked, the greater the possibility of recapture. Thus it can be necessary to boost the number of marked specimens caught at other locations, as we did in the last period of this study. However, we marked relatively few individuals during this study, compared to the total number of specimens caught, and this would be an obvious place to improve a future setup, e.g. by baiting traps with CO<sub>2</sub> when catching specimens for marking.

In the present study we recaptured 2.1% of the marked specimens. This number is higher than found in Lillie et al. [83] where 0.49% were recovered, and in Lillie et al. [82] where 1.5% were recovered, but lower than the study of Brenner et al. [20] where almost 14% of marked specimens were recaptured. As speculated in Lillie et al. [82], the higher recapture percentage of *C. mohave* [20] could be caused by the desert environment lacking obstacles to obstruct the attraction of the traps. We further speculate that the hostile desert environment where *C. mohave* lives can cause specimens to actively search more for breeding sites or host animals and thus make traps more efficient.

In this study we recaptured 29% of the *Pulicaris* group specimens on the pig farm 1,750 m away from the release point (Fig. 1). We tested if the recaptured specimens here had lower fluorescence values than those recaptured in a release point. Removing the lower half of the fluorescence values from the data had no effect on the estimated relative dispersal, indicating that the selected cutoff was robust. Thus the specimens recaptured on the pig farm are regarded as true positives.

The two *Obsoletus* group specimens recaptured in this study were caught in the same location as they were released. Although more recaptures are needed to investigate their dispersal behavior thoroughly, it may reflect a general pattern: As stated in Marquardt et al. [87], species of *Ceratopogonidae* that breed in temporary habitats tend to disperse more broadly than species that breed in more permanent habitats. As showed by Zimmer et al. [136] and Ninio et al. [99], species of the *Obsoletus* group breed in dung and manure inside stables. These breeding sites are more permanent and location-specific than temporary water bodies where the *Pulicaris* group breed [76, 45, 98]. Thus there may be different dispersal patterns for the two species groups.

A concern in this study was that the specimens would die or no specimens would be recaptured during the study, which is why we chose to mark four times

instead of one. The drawback of this approach is that we cannot determine if recaptured specimens in the second, third and fourth periods were marked in the same period they were caught. In this study we assumed that recaptured specimens were released on the nearest release date before recapture. However, it would be more optimal to mark and release only one time during a study period.

An unknown factor in this study is that the *Culicoides* can get in contact with everything in the study area before recapture. If e.g. some types of pollen exhibit autofluorescence, this can cause noise in the data. This is a potential source of bias. In the present study we used unmarked specimens from the study site to establish a cutoff between marked and unmarked specimens. If a source of pollution introduce fluorescence, this will be adjusted for in the cutoff. However, it will also cause weakly marked specimens to be unregistered because their fluorescence will be less than the cutoff.

From the present field experiment it is evident that the vector abundance is higher near host animals (Fig. 1). Traps that are placed far from hosts on agricultural land caught less *Culicoides* than traps near hosts. This conforms with the findings of Rigot et al. [112] who found decreasing numbers of *Culicoides* associated with farms when distance to farms increased.

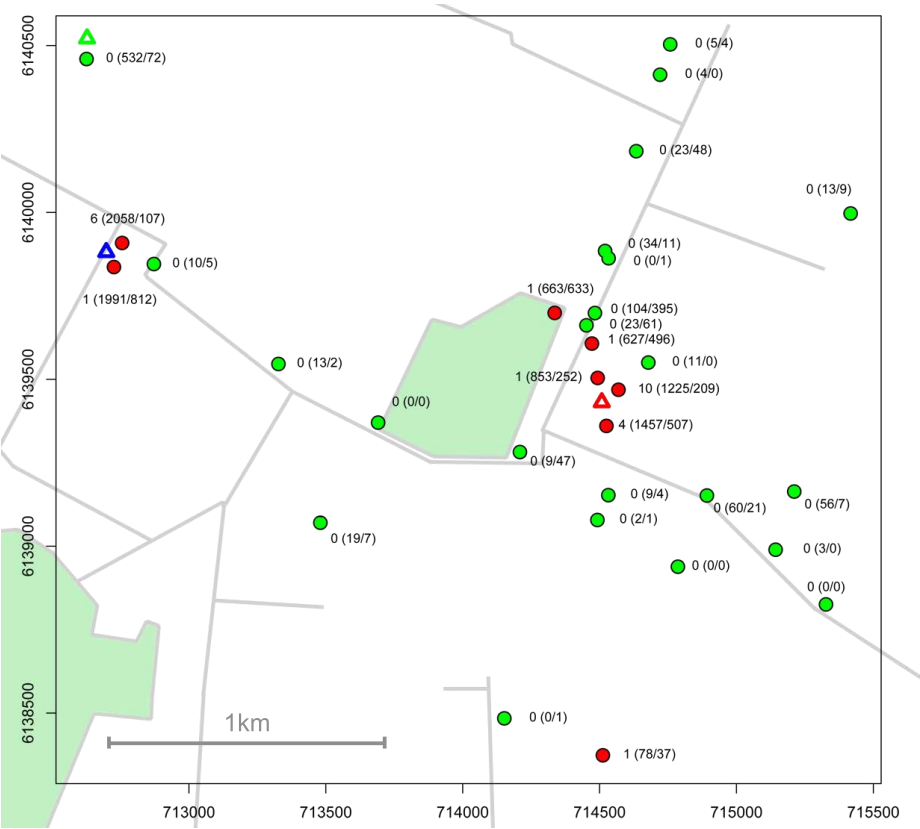
The present technique is a novel tool for the investigation of the dispersal of small flying insects such as *Culicoides*. It has great potential for estimating important parameters for epidemiological models for vectorborne diseases, such as migration between farms as described in the model of Hanski et al. [65], population size as in Trpis et al. [124] and survival rate like Rosewell et al. [113].

## D.5 Acknowledgements

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D.6 Figures

Figure 1



The spatial distribution of the trap catches in the first period in the study (July 22<sup>nd</sup> - July 27<sup>th</sup>). Axes represent the UTM coordinates. The dots represent the trap locations and red dots are locations where *Pulicaris* specimens were recaptured. The numbers at each location represent for this period: *Pulicaris* group specimens recaptured (*Pulicaris* group specimens caught / *Obsoletus* group specimens caught). The triangles show locations of 20 Angus cattle (green), 1700 pigs (blue) and the release point of marked *Culicoides* where 700 cattle were stabled (red). The windrose shows the relative number of time intervals where each wind direction was measured in the first period.

D.7 Tables

Table 1 (next page). The number of marked specimens, captured specimens, marked recaptured specimens (in parentheses), trap catches, number of specimens per trap catch, mean (minimum and maximum in parentheses) wind speed and mean temperature (minimum and maximum in parentheses) measured during the four study periods. Weather variables are measured during the *Culicoides* active periods.

Period	Marked		Captured(recaptured)		Trap catches	Mean no. / trap		Wind speed mean m/s	Temperature mean °C
	Obso.	Puli.	Obso.	Puli.		Obso.	Puli.		
P1	96	274	3749 (2)	9882 (25)	189	20	52	1.4 (0-4.5)	15.2 (10.4-20.1)
P2	234	150	2931	2931 (1)	391	7	7	1.2 (0-3.1)	16.0 (11.7-20.3)
P3	222	378	1829	1110 (2)	236	5	5	0.7 (0-3.6)	15.6 (8.1-19.6)
P4	21	15	581	349 (0)	284	2	1	0.8 (0-2.7)	15.2 (8.7-20.9)
<i>extra</i>	34	136							
<b>Total</b>	607	853	9,090 (2)	14,272 (28)	1110				





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